STUDIES ON THE ALVEOLAR MACROPHAGE

By

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DECLARATION

I confirm that the work described in this thesis is entirely my own. The technical assistance was directly under my supervision.

Signature

Date .....29th October 1979.....

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SUMMARY

This thesis is concerned with (1) the identification of membrane receptors on alveolar macrophages, particularly those for histamine, and (2) some of the metabolic changes which the cell undergoes following incubation with histamine, either in free solution or when bound to particles.

In preliminary experiments, in which some of the variables associated with the "rosette technique" were examined, it was shown that alveolar macrophages bind IgG-coated erythrocytes (EA_G) as well as sheep cells sensitized with IgM and whole serum as a source of complement (EA_M"C3"). Therefore, lung macrophages, like most other leucocytes, appear to bear "immunological receptors" for IgG and complement (presumably C3).

The "rosette technique" was then employed for the identification of histamine receptors on various guinea pig leucocytes using histamine coupled as a rabbit serum albumin conjugate (H-RSA) to formalised ox red cells. It was found that the number of "histamine rosettes" varied from 60-81% for alveolar macrophages, 14-73% for peritoneal macrophages, 14-30% for blood monocytes, 27-48% for lymph node cells, 7-24% for blood lymphocytes and 0-29% for peritoneal and blood neutrophils. Virtually no histamine rosettes were formed with eosinophils or basophils. The percentage of rosette-forming target cells was directly related to the concentration of erythrocyte-bound H-RSA. Free histamine partially inhibited rosette formation by alveolar macrophages in a dose-dependent fashion from 10^{-3} to 10^{-5} moles.l^{-1}, and

complete inhibition was achieved by the H-RSA conjugate. In contrast, amines closely related to histamine such as L-histidine and the major histamine catabolites, imidazoleacetic acid, 1,4-methylhistamine, 1-methyl-4-imidazoleacetic acid and N-acetylhistamine, had no inhibitory effect.

The histamine H1-receptor antagonists, mepyramine and chlorpheniramine, and the H1-receptor agonist, 2-(2-aminoethyl) thiazole, inhibited rosette formation by alveolar macrophages in a dose-dependent fashion. In contrast, the H2-receptor antagonists, burimamide and metiamide, and the H2-receptor agonists, Dimaprit and 4-methylhistamine, were inactive. These experiments suggested that (1) compared to other leucocytes, histamine receptors are particularly well expressed on the alveolar macrophage, (2) these receptors have a high degree of specificity for histamine in that other amines, closely related chemically, did not inhibit rosette formation, and (3) the binding of histamine to the alveolar macrophage membrane is H1- and not H2-receptor dependent.

Experiments were then undertaken to determine whether free histamine, or histamine bound to particles, interacted with its membrane receptor to initiate the "respiratory burst" in alveolar macrophages. Superoxide anion formation (O_2^-) and chemiluminescence, recognized features of the "respiratory burst" of phagocytic cells, were generated by alveolar macrophages following incubation with H-RSA bound to zymosan (H-RSAZ) (and also H-RSA linked to a number of other inert particles such as Sephadex and polystyrene beads).

The degree of O_2^- generation and light emission was comparable to that achieved with serum treated zymosan (STZ). Superoxide formation by alveolar macrophages was related to the concentration of histamine conjugate bound to the zymosan particles as well as the time of incubation. As with histamine rosettes, O_2^- production generated by H-RSAZ in alveolar macrophages also appeared to be dependent on H1-, but not H2-, histamine receptors. Chemiluminescence induced by H-RSAZ was also time- and dose-dependent and totally inhibitible by superoxide dismutase indicating that the O_2^- formation and the burst of light emission are closely related events in the alveolar macrophage. Histamine in free solution did not appear to promote O_2^- generation or chemiluminescence in alveolar macrophages.

Studies were then undertaken in an attempt to determine whether histamine influenced the release of the lysosomal enzyme, β-glucuronidase, following incubation of alveolar macrophages with complement-coated zymosan. Histamine induced a small, but significant, increase in STZ-induced β-glucuronidase release but had no apparent effect on the elaboration of the cytoplasmic enzyme, lactic dehydrogenase. Experiments to determine whether this was an H1- and/or H2-effect were inconclusive. H-RSAZ alone did not appear to influence β-glucuronidase release.

The general conclusion of this work is that there is a direct relationship between histamine and lung macrophages but that for the initiation of the "respiratory burst" histamine requires to be bound to particles to achieve appreciable biological effects. Since histamine is known

to bind to a number of plasma proteins ("histaminopexy") these observations may be of significance both physiologically, in the regulation of oxygen-dependent killing by lung macrophages, and also in disease states where high concentrations of histamine in bronchial secretions may adversely influence the normal functions of alveolar macrophages.

GENERAL INTRODUCTION

In this thesis experiments concerned with the presence of receptors for histamine and the metabolic effects on the alveolar macrophage are described.

As an introduction, the literature relating to the biology of mononuclear phagocytes in general and alveolar macrophages in particular is reviewed.

CONTENTS

CHAPTER I - INTRODUCTION

SECTION A - THE ALVEOLAR MACROPHAGE

1.0 HISTORICAL BACKGROUND

In 1824 Alison described "darkening of the lungs of miners" which was attributed to excessive inhalation of coal dust particles. Virchow (1847) was the first to observe lung cells containing blood pigments from patients with mitral stenosis. Since then many studies have been reported on "particle containing" lung cells but the origin of these materials was for many years unclear (for review, see Bertalanffy, 1964). Some attributed them to an endogenous pigment, whereas others believed them to be the result of inhalation. Knauf (1867), and later Von Ins (1876), conducted inhalation experiments and observed the uptake of dye and dust by cells in the alveolar spaces. Von Ins referred to them as "dust cells". However, it was not until 1884 when Metchnikoff first described the process of phagocytosis that it was appreciated that the material contained in lung macrophages was probably the result of ingestion of foreign material.

In 1924 (Aschoff) the concept of the reticuloendothelial system was developed and included in this "body system" was the macrophage. It was not until relatively recently that there was general agreement to separate macrophages from reticular and endothelial cells and a new classification was introduced on the basis of their common origin, morphology and function. Thus the macrophage cells were grouped together into one system - the Mononuclear Phagocyte System (Van Furth et al, 1972).

Mononuclear Phagocyte System

Cells	Localization
Stem cells	Bone marrow
↓	
Monoblasts	Bone marrow
↓	
Promonocytes	Bone marrow
↓	
Monocytes	Bone marrow Peripheral blood
↓	
Macrophages	Tissues - Connective tissue (histiocytes) Liver (Kupffer cells) Lung (alveolar macrophages) Lymph nodes (free and fixed macrophages) Bone marrow (macrophages) Serous cavities (pleural and peritoneal macrophages) Bone tissue (osteoclasts?) Nervous system (microglial cells)

2.0 ORIGIN AND KINETICS

It has been established that the alveolar macrophage is derived from the haemopoietic stem cell in the bone marrow, released into the blood and transported to the lung as a mononuclear leucocyte (Pinckett et al, 1966; Virolainen, 1968; Godleski and Brain, 1972; Blusse Van Oud Alblas and Van Furth, 1979). Some authors have reported that in the pulmonary compartment, monocytes may undergo a period of maturation before entering the air spaces as alveolar macrophages (Bowden and Adamson, 1972; Vijeyaratnam and Corrin, 1972). Others, however, have found no evidence of cell division or development in the pulmonary interstitium. Both pulmonary alveolar macrophages (located in the alveolar air spaces) and pulmonary tissue macrophages (located in the interstitial tissue) are non-dividing cells (Blusse Van Oud Alblas and Van Furth, 1979). In the steady state, 15% of monocytes leaving the circulation became alveolar macrophages with a life span of approximately one month (Blusse Van Oud Alblas and Van Furth, 1979; Godleski and Brain, 1972). The pulmonary macrophages may be eliminated through the airways (Brain, 1970; Hilding, 1963; Spritzer et al, 1968) or via the lymphatic system (Lauwrerijus and Baert, 1977; Sorokin and Brain, 1975).

The precise kinetics of the monocyte/macrophage system in normal conditions is unclear. It has been reported that alveolar macrophages in pulmonary inflammatory reactions were also bone marrow-derived (Velo and Spector, 1973). Seric substances which stimulated and inhibited monocytopoiesis during experimental peritoneal inflammation have been demonstrated (Van Waarde et al, 1978).

3.0 STRUCTURE AND CHARACTERISTICS

The structure of alveolar macrophages, as shown by light microscopy and metachromatic stains, is highly characteristic. They have considerable heterogeneity in size within the same and different species. The cells are rounded, or slightly oval, with an excentric nucleus. By the light microscope or phase contrast freshly prepared alveolar macrophages have many dark granules of varying size and density. The ultrastructure of lung macrophages (Karrer, 1958) shows many similarities to mononuclear phagocytic cells from other tissues including lymph node, peritoneal cavity, liver etc. (Cohn, 1968). The cytoplasm contains numerous mitochondria, abundant rough endoplasmic reticulum, a prominent Golgi apparatus and many dense lysosomes. When lung macrophages were fixed *in situ* the cell membrane was found to be extensively augmented by microvilli. Phagocytic vacuoles were prominent and contained carbon, iron and other inert particles (Kilburn, 1974).

Mononuclear phagocytic cells can be distinguished and separated from other leucocytes, particularly lymphocytes, by their capacity to adhere to glass surfaces forming mono-layers. Monocytes/macrophages also stain characteristically with non-specific esterases. Under appropriate *in vitro* conditions alveolar macrophages have been shown to divide at a low mitotic rate (Soderland and Naum, 1973).

4.0 METABOLISM

4.1 Energy requirements

In the guinea pig, alveolar macrophages differed from peritoneal macrophages and polymorphonuclear cells in their metabolic requirements (Oren et al, 1963). For instance, the alveolar macrophages derived their energy mainly through aerobic respiration while the other cells studied utilized anaerobic pathways as their primary energy source. This observation applied to both resting and actively phagocytosing cells. In rabbit alveolar macrophages, maximal phagocytic activity occurred only under aerobic conditions and glycolysis played a secondary role (Ouchi et al, 1965). However, other authors have found that in human alveolar macrophages both oxidative and glycolytic energy sources were required for maximal particle uptake (Cohen and Cline, 1971).

4.2 Biosynthetic activity

The synthesis of protein by macrophages has been shown to be associated primarily with the production of digestive enzymes and secretory products (Cohn, 1968; Cohn and Wiener, 1963a,b; Cohn and Benson, 1965a). Synthesis of proteins in the rough endoplasmic reticumum was demonstrated by electron microscopic autoradiography. After assembly the proteins were then transported to the Golgi apparatus and packaged into small Golgi vesicles which represent the primary lysosomes (Cohn et al, 1966). The fusion of this primary organelle with phagosomes forms the secondary lysosomes (Cohn et al, 1966). Following endocytic stimulus, either phagocytic (solid particles) or pinocytic (uptake of soluble), the number of lysosomes in macrophages and their

acid hydrolase content increased (Cohn and Benson, 1965b). This response was related to the type of particle or substance ingested and was abrogated by inhibition of protein synthesis. The increase in enzymatic activity was non-specific since no particular enzyme was induced by a determined stimulus (Axline and Cohn, 1970).

5.0 MACROPHAGE PLASMA MEMBRANE

5.1 Structure

The macrophage plasma membrane consists of a bilayer of phospholipids in which proteins are embedded forming bridges between the cytoplasm and extracellular fluid. This structure has been termed "fluid mosaic membrane" (Singer and Nicolson, 1972). This model system is based on the rotational freedom and translational mobility of individual proteins and lipid molecules (Singer, 1974; Thrasher et al, 1973a). From this concept plasma membranes may be considered as non-uniform structures. Therefore, there may be areas of both poor and rich endocytic activities and areas of different surface receptors which could be regarded as evidence of surface topography. The distribution and mobility of these membrane constituents is not always random and in some circumstances subcellular structures act to direct the movement of particular membrane components (Berlin et al, 1974).

5.2 Surface receptors

5.2.1 Receptors for Fc portion of IgG

Fc receptors have been found in several cells including those of the monocyte/macrophage system. These receptors appear to be "stable" during long term culture conditions (Rabinovitch and De Stefano, 1973a; Gordon and Cohn, 1971). Immunoglobulin can be bound to the cell either free, or in the form of antigen-antibody complexes (Berken and Benacerraf, 1966; Uhr and Phillips, 1966; Arend and Mannik, 1972). When antigen-antibody complexes were used, binding could be detected at lower concentrations of antibody than when

antibody alone was used (Phillips-Quagliata et al, 1971; Arend and Mannik, 1972). The activation of rabbit alveolar macrophages with multiple intravenous injections of complete Freund's adjuvant resulted in an increase in the numbers of receptor sites for IgG per cell (Arend and Mannik, 1973). Rabbit alveolar macrophage Fc receptors were more effectively inhibited by aggregated human IgG than unaltered immunoglobulin (Daughaday and Douglas, 1976).

The chemical nature of Fc receptors is, at present, ill understood. It is stable to treatment with trypsin and other proteolytic enzymes such as papain and pronase but its integrity is apparently dependent on intact sulphydryl and phospholipid structures (LoBuglio and Reinehard, 1970).

It has been demonstrated that alveolar macrophages differ from peritoneal macrophages in the avidity of Fc receptors for immune complexes (Rhodes, 1975). Normal peritoneal macrophages possessed a three-fold greater mean avidity for sensitized erythrocytes than normal alveolar macrophages but the latter showed a greater range of receptor avidities than peritoneal macrophages. In vitro cultures of both macrophage populations increased Fc receptor activities (Rhodes, 1975).

5.2.2 Complement receptors

In human and rabbit alveolar macrophages receptors for C3b and C3d complement components have been identified (Lay and Nussenzweig, 1968; Reynolds et al, 1975).

In contrast to Fc receptors the receptor for the third complement component was destroyed by trypsin and required

Mg^{2+} ions for binding C3-coated particles (Lay and Nussenzweig, 1968).

A different role for IgG and complement receptors on macrophages has been reported. Complement receptors may be primarily involved with particle attachment to the cell and IgG receptors might mediate attachment and ingestion of particles (Griffin et al, 1975a; Mantovani et al, 1972). However, when activated lung and peritoneal macrophages were exposed to C3-coated particles both attachment and ingestion occurred (Shurin and Stossel, 1978; Griffin et al, 1975a).

5.2.3 Surface antigens

The macrophage membrane possesses specific surface antigens which react with the IgG fraction of heterologous anti-macrophage serum (Panijel and Cayeux, 1968; Unanue, 1968; Despont and Crucheud, 1969; Hirsch et al, 1969; Leibovich and Ross, 1975; Holland et al, 1972). Attachment to the macrophage membrane occurred by means of the Fab region (Holland et al, 1972).

Anti-macrophage serum prepared from peritoneal cells selectively inhibited the attachment and ingestion of opsonized erythrocytes and mycoplasma and labelled haemocyanine in vitro, but phagocytosis of polystyrene particles and zymosan was not affected (Holland et al, 1972; Unanue, 1968). Experiments in vitro have demonstrated that in the presence of complement the antiserum is cytotoxic for macrophages (Unanue, 1968). In the absence of complement, antiserum-treated cells showed susceptibility to killing and phagocytosis by untreated normal macrophages, indicating

that membrane changes occurred by which treated cells are no longer recognized by untreated macrophages (Leibovich and Rossi, 1975). However, the anti-macrophage serum cytotoxicity and its ability to inhibit phagocytosis decreased significantly after prolonged incubation (Gallily and Schroit, 1975). The mechanisms involved appear to be a result of shedding of macrophage antigens from the membrane and their subsequent combination with serum, since cells were able to react with fresh anti-serum re-establishing phagocytic inhibition (Schroit et al, 1973).

A rabbit antiserum, specific against rat alveolar macrophages, has also been prepared (Martinez and Montfort, 1973). This antiserum did not react with rat blood monocytes or peritoneal macrophages but showed a high percentage of cross-reaction with rat tissue macrophages, i.e. liver, spleen, lymph node, bone marrow.

5.2.4 Receptors for lymphocytes

Guinea pig alveolar, peritoneal exudate, resident peritoneal and splenic macrophages bound thymocytes and lymph node lymphocytes through their membranes with no evidence of cytoplasm contact as revealed by electron microscopy and phase contrast cinematography studies (Lipsky and Rosenthal, 1973; Salvin et al, 1971). No selectivity for T or B lymphocyte binding was observed. The interaction required viable and metabolically active macrophages, but not lymphocytes, and it was dependent on the presence of divalent cations. The binding was abolished by trypsinization of the macrophages but not of the lymphocytes. The macrophage-lymphocyte interaction

was temperature- and time-dependent with a maximal effect after 60 min of incubation (Rosenthal et al, 1976).

5.2.5 Receptors for T lymphocyte products (MIF)

Macrophage inhibitory factor (MIF) released by T lymphocytes has been reported to act on alveolar macrophages (Warr and Martin, 1973; Reynolds et al, 1974; Moore and Myrvick, 1974).

The interaction of MIF with macrophage membrane was abolished by trypsin or chymotrypsin treatment (David and David, 1972). Neuraminidase destroyed MIF activity indicating that sialic acid groups were probably crucial for its function (Remold and David, 1971). The macrophage membrane MIF "receptors" apparently contain a carbohydrate component since the action of lymphokine was inhibited by both α-LFucose and LFucosidase (Remold, 1973).

Guinea pig peritoneal macrophages incubated with the specific esterase inhibitors, diisopropyl fluorophosphate and soybean trypsin inhibitor showed an increased response to subthreshold doses of MIF (Remold, 1974). A similar enhancement of MIF activity was formed when macrophages were previously treated with a specific plasma esterase inhibitor, α_2-macroglobulin and pure antithrombin III (AT III) but little or no effect with α_1-antitrypsin or C1 esterase inhibitor (Remold and Rosenberg, 1974). These experiments suggested the existence of enzymes on the macrophage membrane which modulate the cell response to MIF.

5.3 Topography of plasma membrane

In rabbit alveolar macrophages, the topography of plasma membrane has been studied, using the transport system

lysine and nucleoside as markers (Tsan and Berlin, 1971a,b; Berlin, 1973). During phagocytosis up to 50% of plasma membrane was internalized and the transport systems remained intact (Tsan and Berlin, 1971b). However, when the membrane structure was disrupted by pretreatment of cells with colchicine or vinblastine the transport system activity decreased during phagocytosis (Ukena and Berlin, 1972; Berlin et al, 1974). These observations led to the conclusion that topographic areas maintained by colchicine- and vinblastine-sensitive structures exist in the macrophage membrane. This structure, probably microtubules, excluded transport carriers from phagocytic areas.

Syngeneic erythrocytes attached to the plasma membrane of murine peritoneal macrophages were not internalized during phagocytosis of latex particles by these cells (Griffin and Silverstein, 1974). These findings reinforce the view that specific areas of plasma membrane may have limited activities.

5.4 Control of macrophage plasma membrane movement

Two systems, the microtubules and the microfilaments, apparently control the phagocyte plasma membrane movements. These structures may be involved in functions of the cells such as chemotaxis, endocytosis and release of chemical products.

The ultrastructure and organization of these systems has been defined (Reaven and Axline, 1973). Microfilaments are found below the plasma membrane. The major portion of microtubules is found in close apposition to organelles and radiate throughout all parts of the cytoplasm, penetrating

the microfilaments and attaching to the plasma membrane (Reaven and Axline, 1973). Microtubules are apparently composed of tubulin protein (Cohn, 1975) and microfilaments of actin and myosin-like proteins (Stossel, 1976). Four different contractile proteins have been isolated from rabbit pulmonary macrophages (Hartwig and Stossel, 1975; Stossel and Hartwig, 1975, 1976).

Studies with substances which alter microtubule (colchicine and vinblastine) and microfilament (cytochalasin B) activities revealed that microfilaments are apparently involved in random locomotion, chemotaxis and phagocytosis. The activation of microfilaments would produce the movement, and activation of microtubules its direction. Thus colchicine-treated cells did not respond to chemotactic stimulus but random movement was increased (Allison et al, 1971). Cytochalasin B inhibition of macrophage spreading, random locomotion, chemotaxis and phagocytosis, but enhancement of lysosomal enzyme release, have been reported (Rabinovitch and De Stefano, 1973b; Allison et al, 1971; Zigmond and Hirsch, 1972; Becker and Showell, 1974; Zurier et al, 1973a). Microtubules may also be important in the release of enzymes from the cells (Becker and Showell, 1974; Zurier et al, 1973b; Ackerman and Beebe, 1975).

In studies on binding of fluorescein Concanavalin A on rabbit alveolar macrophage membrane, a random distribution of the lectin around the cell was found. However, when cells were pretreated with colchicine a capping (aggregation at one pole) of Concanavalin A was observed (Oliver and Berlin, 1976).

It would appear, therefore, that these two structures, microtubules and microfilaments, govern the movements of the cell and maintain the polarity and organization of the plasma membrane.

6.0 ROLE OF THE ALVEOLAR MACROPHAGE IN DEFENCE OF THE LUNG

Alveolar macrophages have been identified as the major defence of the lung against inhaled bacteria or foreign particles (Goldstein et al, 1974; Morrow, 1973; Green and Kass, 1964). In this function of "clearance" the phagocyte must distinguish accurately between foreign material to be removed and the normal tissue constituents. This distinction is probably due to an interaction at the phagocyte's plasma membrane by which various materials are "recognized". Recognition is followed by an appropriate response, i.e. mobilization of macrophages towards the site of invasion, attachment, ingestion and destruction of the particle, all of which are essential events in host defence.

These functions of macrophages in non-specific defence are described below.

6.1 Chemotaxis and chemokinesis

Studies in vivo and in vitro indicate that mononuclear phagocytic cells undergo locomotion, either directional to the attractant (chemotaxis) or non-directional (chemokinesis) (for review see Wilkinson, 1976).

Several substances which attract mononuclear phagocytes in vivo and in vitro have been described. Thus, C5a, a cleavage product of the fifth component of complement (Snyderman et al, 1971), a lymphokine released by stimulated lymphocytes (Ward et al, 1969, 1970; Snyderman et al, 1972; Altman et al, 1973), factors derived from bacteria (Ward, 1968), casein (Wilkinson, 1972) and Corynebacterium parvum (Wilkinson et al, 1973) have been shown to be chemotactic for guinea pig peritoneal macrophages and human monocytes. The rabbit

alveolar macrophage, however, appeared not to respond to agents with chemotactic activity for peritoneal macrophages or polymorphonuclear cells (Ward, 1968).

The way in which these different chemotactic factors stimulated the movement in cells is still poorly understood. Microfilaments and microtubules appear to play a role. Cytochalasin B, which disrupts microfilaments, inhibited locomotion in macrophages (Allison et al, 1971). Colchicine, which depolymerizes microtubules, has been shown to increase random migration of cells but they lost the directionality of movement (Allison et al, 1971).

Divalent cations, Ca^{2+} and Mg^{2+}, may also be important. Thus in media free of these cations, monocyte migration is depressed but not abolished (Wilkinson, 1975).

Substances that increase intracellular levels of cyclic AMP inhibited motility, whereas with agents that raise the levels of intracellular cyclic GMP enhancement has been reported (Estensen et al, 1973). However, no change in intracellular levels of cyclic nucleotides in response to initiation of chemotaxis has been observed (Stossel, 1974).

6.2 Attachment and ingestion

The phagocytic process involves two simultaneous but separate phases, attachment and ingestion (Rabinovitch, 1967). The attachment of the cell to the particle is followed by the formation of microprojections of plasma membrane around the particle and membrane fusion (ingestion).

The attachment may involve non-immunological or immunological mechanisms (Jones, 1975a). The latter are related to the presence of receptors for immunoglobulins

and complement in the macrophage membrane. Little is known about the factor which governs non-receptor-mediated attachment of particles. Physical properties of the particle and of the macrophage membrane may be important. Thus phagocytosis was enhanced when particles were more hydrophobic or when phagocytic cells were more hydrophilic (Van Oss and Gillman, 1972; Thrasher et al, 1973b). Even more, it has been suggested that immunological attachment is governed by the same forces as non-immunological recognition, since opsonizing antibody and complement bound to bacteria or other particles increase their surface hydrophobicity (Vann Oss et al, 1974).

If attachment is followed by ingestion, an invagination of the surface membrane is produced, with an extension of pseudopodia which fuse and completely enclose the particle, forming an intracellular vacuole known as "phagosome". Evidence suggesting that the signal to phagocytose a particle is initiated by the particle itself has been produced. Thus when Fc-coated erythrocytes (EA) attached to macrophages were externally "blocked" (partially opsonized) the ingestion was inhibited, suggesting that a "zipper-like" movement of the membrane around the particle was necessary for phagocytosis (Griffin et al, 1975b, 1976).

Ingestion of particles is an energy-dependent event, presumably necessary for moving the plasma membrane into apposition with the particle. A network of polymerized microfilaments, immediately sub-adjacent to the ingested particle, has been observed (Reaven and Axline, 1973). In rabbit alveolar macrophages, inhibition of endocytosis,

but not attachment by cytochalasin B, has been reported (Malawista et al, 1971). It appears that microtubules are not essential for phagocytosis in guinea pig alveolar macrophages (Ackerman and Beebe, 1975).

The adenylate cyclase system has been associated, in polymorphonuclear and mononuclear cells, with the process of phagocytosis (Manganiello et al, 1971; Bourne et al, 1971; Cox and Karnovsky, 1973). However, in both cells a different effect has been observed. In polymorphonuclear cells, phagocytosis was not accompanied by an increase in cyclic AMP (Stossel et al, 1970; Seyberth et al, 1973; Schmidt-Gayk et al, 1975). Moreover, if cyclic AMP concentration was increased, by addition of drugs, particle uptake was diminished (Stossel et al, 1971; Bourne et al, 1971; Cox and Karnovsky, 1973). In rabbit alveolar macrophages a significant increase in cyclic AMP during phagocytosis of latex bead particles has been observed (Seyberth, 1973). Also, an increase in particle uptake after preincubation of alveolar macrophages with dibutyryl cAMP has been reported (Schmidt-Gayk, et al, 1975). However, other authors have found no increase in rabbit alveolar macrophage cyclic AMP during phagocytosis (Manganiello et al, 1971).

6.2.1 Metabolic changes during phagocytosis

Exposure of phagocytic cells to an appropriate stimuli is followed by a series of co-ordinated metabolic events denominated the "respiratory burst" (Babior, 1978).

In early work, it was demonstrated that phagocytic cells increased their oxygen consumption during phagocytosis

(Baldridge and Gerard, 1933). Later it was shown that most of the oxygen uptake by polymorphonuclear cells during phagocytosis was converted to hydrogen peroxide, an agent with a known bactericidal capacity (Iyer et al, 1961). The authors related the respiratory burst with the microbicidal activity of the cells (Iyer et al, 1961). These events, first described in polymorphonuclear cells, have also been demonstrated in mononuclear phagocytes including alveolar macrophages (Oren et al, 1963; Ouchi et al, 1965; Romeo et al, 1973b; Rossi et al, 1975; Klebanoff and Haman, 1975). The metabolic burst is stimulated by only attachment, or perturbation by surface agents, of the phagocytic cell membrane. No ingestion of the particle is required (Graham et al, 1967; Romeo et al, 1973a; Goldstein et al, 1975).

Simultaneously with the increase in oxygen consumption and hydrogen peroxide production, an increase in superoxide radical activity and in glucose oxidation has been demonstrated. Both metabolic pathways of glucose oxidation were increased. However, in lung macrophages the hexose monophosphate shunt (HMP)/tricarboxylic acid cycle activity ratio was lower than in peritoneal macrophages and polymorphonuclear cells, which is consistent with their higher content of mitochondria (Rossi et al, 1975). The initial events of metabolic activity in all mononuclear phagocytes was characterized by an enhancement of the HMP activity (West et al, 1968; Rossi et al, 1975).

The stimulation of the enzyme NADPH oxidase might initiate the respiratory burst (Fig. 1). The increase in

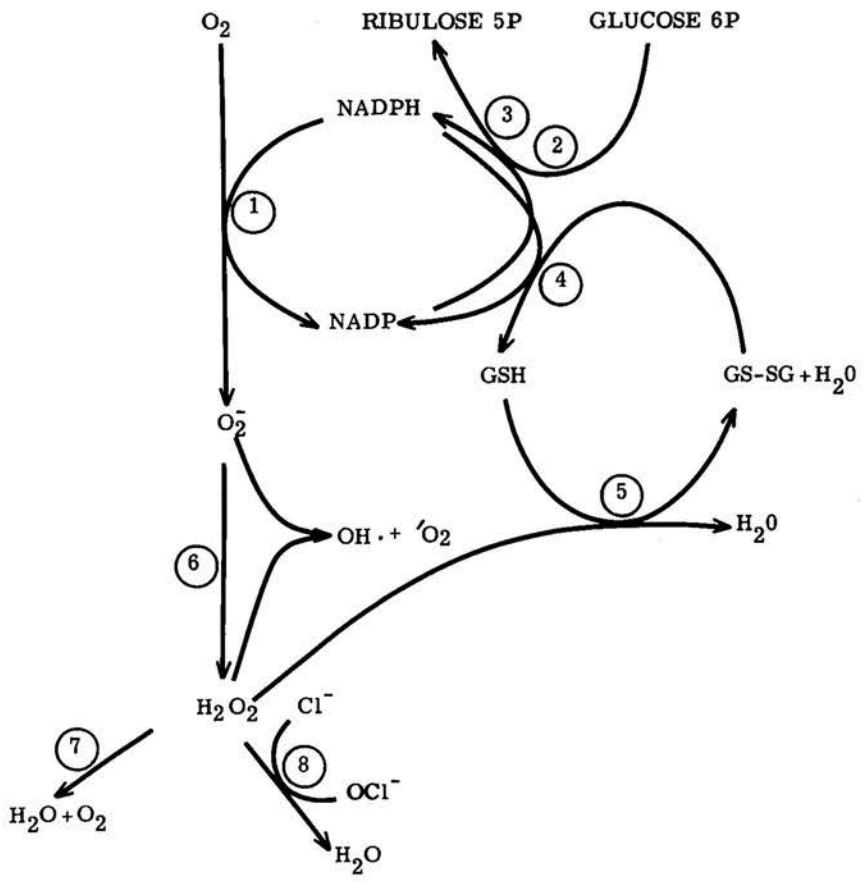


Fig. 1

Enzymes and products of the "respiratory burst" in phagocytic cells. (1) Nicotinamide adenine dinucleotide phosphate oxidoreductase; (2) glucose-6-phosphate dehydrogenase; (3) 6-phosphogluconate dehydrogenase; (4) glutathione reductase; (5) glutathione peroxidase; (6) superoxide dismutase; (7) catalase; (8) peroxidase. $O_2^{-\cdot}$, superoxide radical; H_2O_2, hydrogen peroxide; $OH^{\bullet}$, hydroxyl radical; $^1O_2$, singlet oxygen; OCl^-, hypochlorite anion. (Modified from P. Beswick, Ph.D. Thesis.)

HMP is probably secondary to the increase in NADP. There are two sources for NADP. The O_2^- forming system in which NADPH is the electron donor and the glutathione peroxidase-glutathione reductase system (Vogt et al, 1971).

The amount of hydrogen peroxide found in macrophages is only a small percentage of O_2 consumed (Rossi et al, 1975). Several systems of hydrogen peroxide disposal have been described in mononuclear cells, catalase (Gee et al, 1970), glutathione cycle (Vogt et al, 1971), a peroxidase, similar but not identical to the myeloperoxidase of neutrophils (Romeo et al, 1973c) and the peroxidation of unsaturated fatty acids (Mason et al, 1972). All these systems may be responsible for the high rate of hydrogen peroxide disappearance observed in these cells. In alveolar macrophages the glutathione cycle has been described to be quantitatively more important (Gee et al, 1971).

6.2.2 Role of the "respiratory burst"

The increase in oxygen consumption and glucose oxidation in phagocytic cells may provide the energy (ATP) necessary for their activities and movements. On the other hand, the metabolic burst produces metabolites with microbicidal and cytotoxic properties. Thus Klebanoff (1967a,b) described the "triad" of hydrogen peroxide, myeloperoxidase and a halide (Cl^-, I^-) to be highly bactericidal. This system first described in polymorphonuclear cells, has also been reported to operate in alveolar macrophages (Paul et al, 1973). In addition, superoxide radical, singlet oxygen and hydroxyl radical might also play a bactericidal and cytotoxic role (Babior et al, 1973;

Babior et al, 1975; Krinsky, 1974; Simchowitz and Spilberg, 1979).

6.3 Degranulation

During phagocytosis, the metabolic events which were initiated in the outer surface of the membrane, are internalized when the invagination is produced. This newly formed vacuole fuses with the lysosome membrane which secretes its enzymatic content into its lumen. This process has been termed "degranulation".

The fusion of the phagosome with the lysosome is apparently an essential microbicidal mechanism. Thus studies in vitro have demonstrated a survival of Mycobacterium tuberculosis, Listeria monocytogenes and Toxoplasma gondii inside the phagocytic cells, when the fusion of lysosome with the phagocytic vacuole was inhibited (Armstrong and D'Arcy Hart, 1971; Jones, 1975b).

In peritoneal and alveolar macrophages the presence of several lysosomal hydrolytic enzymes has been reported (i.e. β-glucuronidase, acid phosphatase, cathepsin, acid ribonuclease, esterase, lipase) (Cohn and Wiener, 1963a). In vitro, the release of lysosomal enzymes into the culture medium during phagocytosis of different particles has been observed (Cohn and Wiener, 1963b; Cardella et al, 1974; Davies et al, 1974a; Ackerman and Beebe, 1974). It has been suggested that this phenomenon may also occur in vivo, producing an inflammatory reaction (Davies and Allison, 1976), since the release of lysosomal enzymes has been observed during phagocytosis of substances known to produce chronic inflammation (Davies et al, 1974b; Page et al, 1974).

The role of microtubules, microfilaments and cyclic nucleotides in regulating the process of degranulation has been studied. Cytochalasin B inhibited phagocytosis and increased enzyme release in alveolar macrophages (Ackerman and Beebe, 1975). Colchicine acting on microtubules inhibited both phagocytosis and degranulation (Ackerman and Beebe, 1975). The same authors found that cyclic nucleotides and their dibutyryl analogues had no appreciable effect on β-glucuronidase release by guinea pig alveolar macrophages. However, in mouse peritoneal macrophages, an inhibition with cyclic AMP and an increase with cyclic GMP of lysosomal enzyme release has been reported (Weissmann et al, 1971b).

7.0 SECRETORY FUNCTION OF MACROPHAGES

The precise role of macrophages (including alveolar macrophages) in chronic inflammatory reactions is largely unknown. The capacity of these cells to influence the surrounding medium might be due to the production and secretion of several chemical mediators. In addition to lysosomal enzymes secreted during phagocytosis of different substances and by stimulation of T lymphocyte products (Pantalon and Page, 1975), macrophages are able to release other chemical substances with probable participation in inflammatory reactions. These substances are analysed below.

7.1 Neutral proteinases

It has been demonstrated that macrophages are able to secrete several neutral proteinases that may play an important role in both the destruction and repair phases of the chronic inflammatory process. These enzymes include:

(a) Plasminogen activator which catalyzes plasminogen to plasmin, a potent fibrinolytic enzyme. It is induced during macrophage activation by non-specific stimulus, i.e. latex phagocytosis, Concanavalin A, thioglycollate, phorbol myristate (Unkeless et al, 1974) or by immunologically specific stimulation by a T lymphocyte product (Gordon et al, 1978).

(b) Collagenase is present in normal alveolar macrophages (Senior et al, 1972) but only in stimulated peritoneal macrophages (Wahl et al, 1974). Its secretion can be induced in vitro culture by exposing the cells to bacterial lipopolysaccharide (Wahl et al, 1974).

(c) Elastase has also been demonstrated to be synthesized and released by macrophages including alveolar macrophages (Ackerman and Beebe, 1974). This enzyme may play an important role in pulmonary pathology.

7.2 Lysozyme

Lysozyme is synthesized and secreted by several mononuclear phagocytic cells (Heise and Myrvik, 1967; Gordon et al, 1974; McClelland et al, 1975). It may have a bactericidal role and possibly destroys dead bacterial cell walls.

7.3 Complement components

The snythesis of C2 by guinea pig peritoneal macrophages (Wyatt, 1972) and C4 by guinea pig peritoneal lung, and liver mononuclear cells, has been reported (Littleton et al, 1970).

7.4 Other products

Other products secreted by macrophages include pyrogen (Hahn et al, 1967) and interferon (Acton and Myrvik, 1966).

7.5 Summary

In summary, macrophages under different stimuli have shown the capacity of synthesis and secretion of several chemical mediators. Through these products, macrophages may be able to modify their surrounding medium and participate in functions such as specific and non-specific immunity and in some pathological conditions such as chronic inflammatory reactions.

8.0 ROLE OF THE ALVEOLAR MACROPHAGE IN SPECIFIC IMMUNITY

It is well established that macrophages participate in the production of antibody and in the regulation of lymphoid cell functions, but the precise mechanism of these complex interactions is not understood (for recent review see Nelson, 1976).

Most of the work has been with macrophages other than those from the respiratory tract (i.e. spleen, lymph node, peritoneal cavity). The role of the alveolar macrophage in these immunological mechanisms has received relatively little attention. However, the lung has been recognized as an organ capable of mounting a local immune response following exposure to antigen (reviewed by Kaltreider, 1976).

A comparison of the alveolar macrophages with other mononuclear phagocytes in terms of specific immunity is discussed below.

8.1 Role of macrophages in antibody-mediated immunity

Several studies *in vivo* and *in vitro* have demonstrated that macrophages from the spleen, peritoneal cavity and lymph nodes are required for the development of a primary antibody response (Sabin, 1939; Fishman, 1959; Gallily and Feldman, 1967; Unanue, 1972; Katz and Benacerraf, 1972). *In vitro*, the macrophages appear to participate mainly in response to T cell-dependent antigens. Thus certain antigens, i.e. bovine serum albumin (Mitchison, 1969), haemocyanine from Maia squinado (Unanue and Askonas, 1968a), sheep or burro erythrocytes (Mosier, 1967; Pierce, 1969; Shortman and Palmer, 1971), viruses (Fishman, 1961; Bluestein and Pierce, 1973), might be considered as

macrophage- as well as T cell-dependent. At the present time the "helper" role of macrophages in antibody production can be summarized as follows: (1) antigen modification; (2) direct contact with lymphocytes, and (3) indirect influence on lymphocytes by the release of macrophage-derived chemical mediators. These putative functions are discussed individually below.

8.1.1 (a) Antigen modification ("handling") by macrophages

Studies in vitro have demonstrated that macrophages were able to "take up" and modify a number of different antigens (Unanue, 1972). Most of the radiolabelled antigens which penetrate the macrophage were destroyed. Some escaped degradation and were released from the cells (Unanue and Askonas, 1968b). Small quantities of undegraded antigen adhered to the macrophage membrane and were capable of reacting with specific antibodies (Unanue et al, 1969). This macrophage-bound antigen was shown to be highly immunogenic. Furthermore, macrophage-bound antigen evoked a primary antibody response in vitro (Pierce et al, 1974; Seeger and Oppenheim, 1970).

Soluble antigens which are potentially tolerogenic can be rendered immunogenic after uptake and concentration by macrophages. Thus macrophages may appear to have the capacity to eliminate "tolerogenicity" (Mosier, 1975).

8.1.1. (b) "Handling" by alveolar macrophages

The role of the alveolar macrophage in antibody formation is less clear. Studies in vitro suggested that alveolar macrophages from the dog were able to process

certain antigens, i.e. ragweed and keyhole limpet haemocyanide, in a comparable way to that described for other macrophages (Pruzanski et al, 1976). That is to say, a certain amount of material remained membrane-bound. On the other hand, the complete degradation of bacterial antigens, with loss of its immunogenic capacity, by BCG-stimulated rabbit alveolar macrophages was reported (Cohn, 1964, 1968). These findings are not necessarily mutually exclusive since the experimental conditions were different. Thus the interaction of the alveolar macrophage with putative antigens might result either in complete elimination or "immunogenicity"; pathological lung reactions might be a consequence of a failure of such mechanisms (Mackaness, 1971).

8.1.2 Macrophage-lymphocyte interaction

The sequence of events in the interaction between macrophages and lymphocytes in the stimulation of the antibody response remains controversial. One view is that macrophage-bound antigen triggers specific T cells which in turn become activated to regulate the expression of a B cell response to that antigen (Unanue, 1972; Katz and Unanue, 1973). Alternatively, antigens might first interact with T cells (which release a T cell immunoglobulin, IgT) which are cytophilic for macrophages. IgT bound to macrophages would then stimulate B cells for specific antibody production (Feldman, 1972). In both situations macrophages are apparently essential. Thus the interaction between macrophages, T cells, B cells and antigens is an absolute requirement for development of an optimal antibody response in vitro (Pierce, 1973; Unanue, 1972; Claman and Mosier, 1972; Katz and Benacerraf, 1972).

The macrophage-lymphocyte interaction has been shown to occur both by physical contact between these cells and also by release of chemical mediators from the macrophages.

Several different macrophages, including those from the respiratory tract, are able to interact physically with both T and B lymphocytes either in the presence or absence of antigen. Ultrastructural studies revealed a broad area of cell to cell contact (Lipsky and Rosenthal, 1973) suggesting a receptor-like mechanism (discussed in relation to macrophage membrane).

8.1.3 Macrophage-derived factor

Several macrophage-derived soluble factors, able to modify the antibody response, have been described. These chemical mediators help lymphocytes or replace the need for macrophages in lymphocyte cultures. These factors can act on the antigen or on lymphocytes.

8.1.3 (a) Macrophage-derived factor acting on antigen ("solubilizing" factor)

This factor has been identified in supernatants of peritoneal macrophage cultures. The supernatant was able to restore the immune response of mouse spleen cells to sheep erythrocytes (SRBC) previously depressed by removal of macrophages (Hoffman and Dutton, 1971; Calkins and Golub, 1972; Wood and Gaul, 1974). Also, macrophage supernatant-treated sheep red cells were able to stimulate the production of anti-sheep red cell antibody in the absence of macrophages or supernatant (Hoffman and Dutton, 1971). This macrophage-derived factor appears to act by "solubilizing" SRBC and conferring them with an immunogenic capacity, since

"solubilized" SRBC by sonication acted in the same way (macrophage-independent) (Feldman and Palmer, 1971). This macrophage-derived factor might be associated with lysolecitine released into the culture medium by activation of phospholipase A. This enzyme is known to be present in large amounts in stimulated macrophages (Rosenstreich and Oppenheim, 1976; Munder and Modolell, 1973). Lysolecitine and phospholipase are both highly membrane active substances and are able to produce red cell lysis (Seljelid, 1975).

8.1.3 (b) Macrophage-derived factors acting on lymphocytes

8.1.3 (b)(i) Viability promoting factor

Studies in vitro have demonstrated that peritoneal exudate macrophages or adherent cells from the spleen enhanced the survival of lymphoid cells in culture and had an essential participation in the antibody response to sheep red cells (Shortman and Palmer, 1971). This effect could be substituted by 2-mercaptoethanol indicating that a soluble factor derived from macrophages was probably acting on lymphocytes. Therefore, macrophages might be able to play a trophic role in supplying factor(s) which may maintain lymphoid cell viability (Chen and Hirsch, 1972).

8.1.3 (b)(ii) Lymphocyte activating factor (LAF) or mitogenic protein (MP)

It was shown that cultures of mouse peritoneal exudate macrophages and human leucocytes secreted a factor which, in the presence of antigen or mitogens, potentiated cell division of thymocytes and to a lesser extent T and B

lymphocytes. It has been termed lymphocyte activating factor (Gery et al, 1972) or mitogen protein (Unanue, 1978). This substance(s) has been characterized and its function studied (Unanue, 1978). It is a low molecular weight protein with no apparent enzymatic activity. It is heat labile (Gery and Handschumacher, 1974; Unanue, 1978). The release of this factor(s) was increased by stimulation with endotoxin PHA or irradiation of macrophages in vivo (Geiger et al, 1973). Highly pure preparations of "mitogenic protein" stimulated proliferation of thymocytes in the absence of antigens (Calderon et al, 1975).

8.1.3 (b)(iii) Macrophage-derived factor acting on B cell differentiation

Spleen cells from immunized mice, incubated with macrophage culture fluid, differentiated into plaque forming cells in the absence of antigen and T cells (Calderon et al, 1975). This finding was interpreted as representing immune (memory) B cells differentiating into antibody-secreting cells in the presence of a macrophage-derived factor.

8.1.3 (b)(iv) Factor increasing or suppressing antibody production in vitro

Another factor found in macrophage culture fluid enhanced or decreased antibody production when added to spleen lymphoid cells in vitro. This activity required immune spleen cells and the same antigen used for priming T and B cells (Calderon et al, 1975; Unanue, 1978). In these experiments spleen cells from mice immunized several weeks before harvesting for in vitro culture responded by an increase in antibody production. In freshly primed mice the antibody response could be enhanced or suppressed

depending upon the amount of macrophage culture fluid. Therefore, this macrophage-derived factor increased or decreased antibody production following incubation with antigen, although the magnitude of these modifications was dependent upon the immune status of the spleen cells.

8.2 Role of macrophages in cell-mediated immunity

Macrophages are also required for the maturation or development of cell-mediated immunity *in vivo* and *in vitro* (Bloch and Nordin, 1960; Seeger and Oppenheim, 1972; Wahl *et al*, 1975). As in antibody production, macrophages are also intimately involved in antigen binding and activation of T lymphoid cells for the production of mediators by the specifically sensitized cell (Rosenstreich and Oppenheim, 1976). T lymphocytes, after stimulation with specific antigen or mitogen, release biological mediators (or lymphokines) with a great variety of effects, e.g. attraction and activation of macrophages. These interactions among T lymphocytes and macrophages confer on the latter a non-specific increase in their anti-bacterial, anti-viral and anti-tumoural capacities and induce them to secrete biologically active substances. Although the activation of macrophages requires a lymphocyte-dependent specific antigen recognition, macrophages acquire a non-specific activity (Krahenbuhl and Remington, 1971; Remington *et al*, 1972; Simon and Sheagren, 1971, 1972; Mackaness, 1969).

8.2.1 Role of macrophages in lymphocyte responses to mitogens in vitro

Mitogens, such as phytohaemagglutinin (PHA) and Concanavalin A (Con A) are able to stimulate T lymphocyte

proliferation *in vitro*. In macrophage-depleted lymphocyte cultures a reduced, but not absent, response to PHA and Con A has been observed. However, it was markedly enhanced by the re-addition of mononuclear phagocytic cells (Lorhmann *et al*, 1974). If highly purified T lymphocytes were stimulated with mitogens, in the absence of macrophages, no detectable response was observed. On the other hand, activity was restored by the addition of syngeneic macrophages or their supernatant. Although mitogens were able to bind T cells, the proliferation did not occur unless macrophages or a macrophage-derived soluble factor was present. Furthermore, macrophage-bound PHA was also capable of stimulating purified T lymphocytes (Rosenstreich and Oppenheim, 1976). Therefore, lymphocytes can be activated directly by macrophage-bound mitogens or through a two "step" process, i.e. the binding of mitogen to lymphocytes and the activation through the lymphocyte activating factor (LAF) previously described (Rosenstreich and Oppenheim, 1976).

8.2.2 Alveolar macrophages and lymphocyte response to mitogens in vitro

The role of the alveolar macrophage in lymphocyte response to mitogens has not been studied extensively and the work which has been reported has used several different species. For instance, guinea pig alveolar macrophages greatly enhanced the response of lymph node lymphocytes to PHA and Con A (Gorenberg and Daniele, 1978). When highly purified alveolar macrophages, resident peritoneal and glycogen-induced peritoneal macrophages were compared in

their effect as accessory cells in mitogen-stimulated lymphocyte proliferation, the alveolar macrophage showed a higher stimulatory effect than both types of peritoneal macrophage. Furthermore, the presence of alveolar macrophages in the cell culture was not necessary since if both populations (lymphocytes and alveolar macrophages) were separated by a filter, a stimulatory effect was also obtained. This suggested that the effect was produced through a soluble diffusible factor. However, when peritoneal cells were studied, its presence in the cell culture was required, i.e. the effect could not be obtained with factor alone indicating a qualitative as well as a quantitative difference in the activating factor in both macrophage populations (Gorenberg and Daniele, 1978).

In contrast, in rats and dogs a factor with a suppressor effect on lymphocyte mitogen-stimulated proliferation from purified alveolar macrophages has been described (Holt, 1979a; Ansfield et al, 1979). When canine cells were studied a decreased response to mitogens of lung lymphocytes compared to blood lymphocytes was observed. This inhibitory effect was due to both an inherent hyporesponsiveness of lung lymphocytes and a suppressor effect of alveolar macrophages. The same inhibitory effect was obtained when blood lymphocytes were mitogen-stimulated in the presence of alveolar macrophages (Ansfield et al, 1979).

In the rat, two different populations of alveolar macrophages have been described; one strongly adherent which also had an inhibitory effect on mitogen-stimulated

lymphocyte proliferation and another weakly adherent population which stimulated lymphocyte proliferation to mitogens (Holt, 1979b). These data are not necessarily contradictory since both stimulatory and inhibitory activity in lymphocyte proliferation by other macrophage supernatants have been previously described (Calderon and Unanue, 1975; Calderon et al, 1974; Keller, 1975; Nelson, 1973).

9.0 MACROPHAGE ACTIVATION

The concept of macrophage activation was first introduced to indicate the adaptive changes produced in those cells which enable them to express enhanced resistance to infection. However, not only anti-bacterial properties are increased in activated macrophages. They also show augmentation in anti-viral and anti-tumour activities as well as secretion of biological substances. These latter products are not normally secreted, i.e. plasminogen activator, collagenase and elastase (Gordon, 1977; Gordon et al, 1978).

Activated peritoneal macrophages showed morphological, biochemical and physiological changes. They exhibited increased adherence, spreading and ruffled membranes (Blanden, 1968), decreased membrane permeability (Diengdoh and Turk, 1967), increased pinocytic (Edelson et al, 1975) and phagocytic capacities (Bianco et al, 1975) and an increased number of phagolysosomes and endocytic vesicles (Cohn and Benson, 1965c). Stimulation of metabolism includes an increase in glucose oxidation and oxygen consumption increase in hexosemonophosphate shunt (Nathan et al, 1971) and NADPH oxidation (Poulter and Turk, 1975a,b), and an augmentation of superoxide anion production (Johnston et al, 1978). However, no increase in hydrogen peroxide was reported (Nathan and Root, 1977), an observation which may be related to the relatively high content of hydrogen peroxide disposal system in macrophages.

Inflammatory macrophages obtained from the peritoneal cavity had increased amounts of lysosomal enzymes within

the large phagolysosomes (Cohn and Benson, 1965c; Cohn, 1968; Cohn, 1978). Also, BCG-activated alveolar macrophages exhibited increased levels of acid phosphatase, lysozyme and lipase as compared to alveolar macrophages from unstimulated rabbits (Cohn and Wiener, 1963a). In vitro, lymphokine-activated macrophages had no increase in β-glucuronidase activity after 1 hr contact (Poulter and Turk, 1975a). However, an increase in this enzyme was observed after 3 days contact (Poulter and Turk, 1975b) suggesting that there are subtle sequences in activation.

Another property of activated macrophages is their capacity to recognize and destroy neoplastic cells. Such activated macrophages can be obtained in vivo from animals infected with bacteria (BCG or Listeria) or certain protozoan (Toxoplasma gondii) infections or from animals stimulated with complete Freund's adjuvant or killed Corynebacterium parvum (Hibbs et al, 1972; Remington et al, 1975; Meltzer et al, 1975). In vitro macrophages can be activated and induced to be cytotoxic to tumour cells by a product of sensitized T cells secreted in the presence of sensitizing antigen (Piessens et al, 1975; Lohmann-Nattes, 1976). This macrophage cytotoxic factor (MCF) was shown to bind allogeneic and syngeneic macrophages rendering them cytotoxic (Evans et al, 1972; Zembale et al, 1973).

The functional criteria frequently used to distinguish normal from activated macrophages have been the increased capacity of killing of intracellular organisms (Mackaness, 1962, 1964), inhibition and/or killing of tumour cells

(Evans, 1975) and inhibition and/or stimulation of lymphocyte proliferation (Nelson, 1973; Keller, 1975). There is some evidence, however, that activated macrophages characterized by one functional criterion do not always possess the other functional characteristic as well (Wing et al, 1977). Thus some expressions of activation can be manifested before others. This may indicate that activation occurs in "steps" and that the number of these steps is determined by the appropriate activating factor which in turn decided the particular biological role (North, 1978).

In summary, macrophages play an important role in the induction of the immune response and are a principal effector cell in cell-mediated immunity. Also, they actively participate in anti-bacterial, anti-tumoural and anti-viral defence of the organism.

The role of the alveolar macrophage in the immunological response is poorly understood. However, it is reasonable to assume that its influence is almost certainly comparable to other well known activities in defence against bacteria in the lungs, since airways are constantly invaded by a great variety of antigens.

SECTION B - HISTAMINE

1.0 HISTAMINE

Histamine (2-aminoethylimidazol) is a low molecular weight pharmacological agent formed in most tissues. It is stored within mast cells or blood basophils as a heparin protein complex in metachromatic granules of these cells. It is released by a wide variety of specific and non-specific stimuli. These include cold, trauma, burns, infection and in antigen-antibody reactions mediated by homocytotropic antibody. Certain substances, i.e. compound 48/80, release histamine by their selective interaction with the mast cell membrane.

1.1 Synthesis and metabolism of histamine

Histamine is synthesized by the decarboxylation of the amino acid L-histidine by the action of the enzyme, histamine decarboxylase (Fig. 2). In several species including man, guinea pig, rat and rabbit, histamine is metabolized by two routes. However, the importance of these routes varies among species. In one pathway, histamine is methylated to 1,4-methylhistamine by the enzyme, histamine N-methyltransferase. Methylhistamine is subsequently deaminated by monoaminoxidase to form 1,4-methyl imidazole-acetic acid. In the other pathway, histamine is deaminated by diaminoxidase ("true histaminase") to form imidazole-acetic acid which can be excreted or previously conjugated as ribosyl imidazoleacetic acid. N-methyltransferase is associated with mononuclear cells whereas "true histaminase" is present predominantly in granulocytes (Zeiger et al, 1976).

Studies with labelled histamine have shown that the body has an enormous capacity to degrade histamine. It

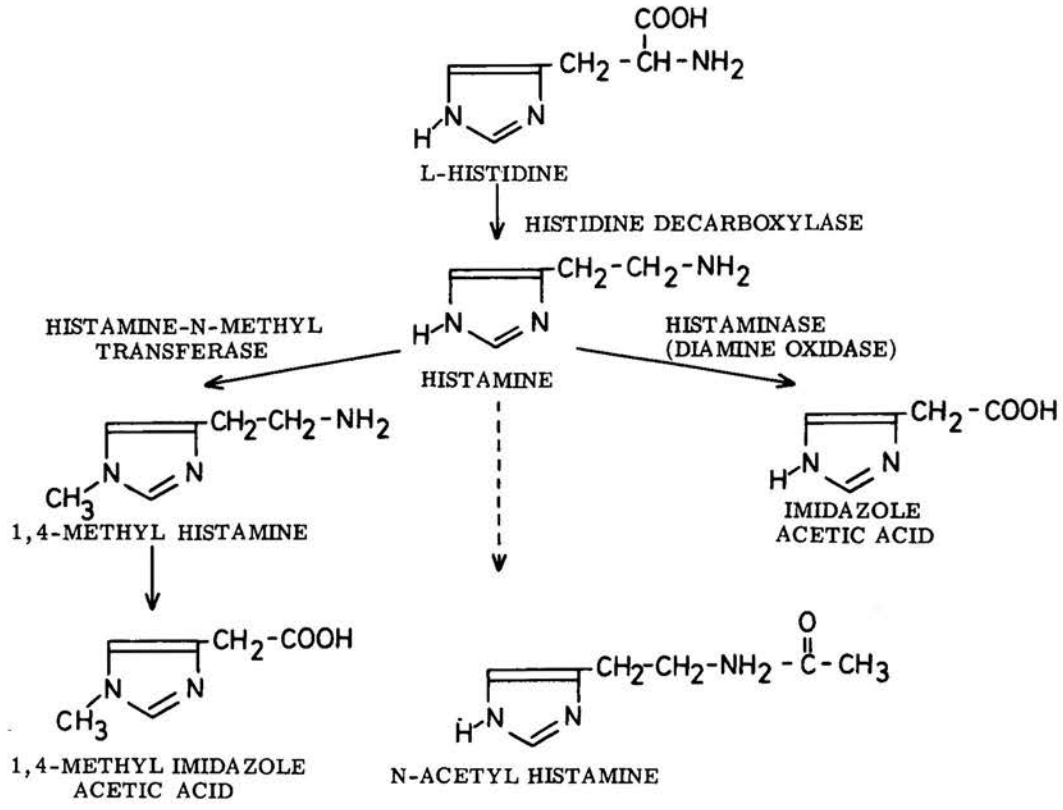


Fig. 2

Pathways of synthesis and metabolism of histamine in man (after Schayer, 1959).

has largely disappeared from plasma and appeared in almost all tissues as metabolites within minutes after intravenous administration. Only a small percentage of the labelled histamine was formed unchanged in the urine (Schayer, 1959).

1.2 "Histaminopexy"

Studies in vitro in human and rat serum have demonstrated that histamine had the capacity to bind to a small polypeptide of 1,000-5,000 M.W. The amine attached predominantly to albumin from which it could be liberated after papain digestion or during the process of coagulation (Gecse et al, 1972). This phenomenon called "histaminopexy" may have relevance to the results described later in this thesis.

1.3 Histamine antagonists

Since histamine has been shown to participate in a number of physiological and pathological states there have been numerous studies on the development of histamine antagonists. The first group of histamine antagonists (pyrilamine maleate, diphenhydramine, mepyramine, chlorpheniramine, etc.) were able to "block" only some of the actions of histamine. Later these drugs were designated H1 histamine antagonists (Ash and Schild, 1966). It was not until 1972 that a second group of histamine antagonists, H2, were discovered (burimamide, metiamide, cimetidine) (Black et al, 1972) which antagonised histamine effects unaffected by the H1 antagonists (or "classic anti-histamines"). Thus at the present time it is thought that the activities of histamine are mediated by two different types of tissue receptors (H1 and H2), the ratio of distribution of both types of receptor varying in different organs (Black et al, 1972; Chand and Eyre, 1975).

1.4 Role of histamine

The first pharmacological activity of histamine was reported by Dale and Laidlaw in 1919. They observed that when this drug was applied locally it produced an inflammatory reaction with redness, swelling and oedema. Following injection it produced "anaphylactic-like" symptoms, i.e. hypotension, tachycardia, increased capillary permeability with loss of plasma through the capillary epithelium and haemoconcentration accompanied by a fall in body temperature. Histamine also was found to produce an increase in gastric and pancreatic secretion and constriction of smooth muscle of bronchus, stomach, duodenum and urinary bladder. Another role proposed for histamine is in regulation of the microcirculation (Schayer, 1965). According to this view, histamine would be synthesized, but not stored, continually in the small blood vessels and upon accumulation relaxes the precapillary sphincters. Cyclic accumulation of histamine and "wash out" would account for the spontaneous opening and closing of small blood vessels in the vascular bed.

Histamine synthesis has been found to be increased in tissues undergoing rapid growth or repair such as the foetus, regenerating liver, granulating tissue of wounds and in certain tumours (Kahlson and Rosengren, 1968).

The participation of histamine in hypersensitivity reactions is well known. In these pathological conditions it is released with other mediators (slow reacting substance of anaphylaxis (SRS-A) and eosinophil chemotactic factor of anaphylaxis (ECF-A)) when mast cells or basophils

specifically sensitized with IgE (or equivalent tissue-sensitized antibody) interact with antigen.

Further studies have shown that histamine may apparently function as a regulator as well as a mediator of hypersensitivity. Thus histamine inhibited histamine release from basophils probably by increasing cyclic AMP (Bourne et al, 1971), an effect mediated through the H2-receptors (Lichtenstein and Gillespie, 1973). In addition, ECF-A (Kay and Austen, 1971) and histamine (Turnbull and Kay, 1976; Jones and Kay, 1977) will promote directional migration of eosinophils in vitro and their accumulation to skin sites in vivo. Eosinophils in turn have a regulatory role by their histamine inhibitor content and their inhibition of mast cell regranulation (Jones and Kay, 1976). Also, histamine induced the release of an eosinophil immobilizing factor from non-adherent mononuclear cells (Kownatzki et al, 1977).

A probable regulatory role of histamine in the immune response by increasing cyclic AMP in immunological cells, mainly through an H2-receptor, has been reported (Bourne et al, 1974). Thus an inhibition of antibody production (Melmon et al, 1974), antigen-induced lymphocyte proliferation (Artis et al, 1975), release of MIF (Rocklin, 1976) and lymphocyte-mediated cytotoxicity (Henney et al, 1972) have been demonstrated. Also interesting was the report that lymphocytes increased their functional histamine receptors with maturation (Roszkowski et al, 1977) and after primary immunization (Plaut et al, 1973b).

Histamine may also play a role in acute inflammatory reactions by inhibiting the enzyme release from poly-

morphonuclear cells (Zurier et al, 1974; Busse and Sosman, 1976).

Histamine, therefore, has important physiological and pathological roles. Its participation in inflammatory and hypersensitivity reactions is well known. On the other hand, its role in the immune response needs further investigation.

The principal actions of histamine are summarized in Table I.

Action	Species	Receptor Mediated	Reference
Increases gastric secretion	Human; dog; rat; guinea pig	H2	Ash and Schild, 1966; Black et al, 1972
Causes contraction of ileum	Guinea pig	H1	Ash and Schild, 1966
Increases vascular permeability	Human; guinea pig; rat; dog	H1 & H2	Halpern, 1976; Grennan et al, 1975
Causes contraction of tracheal and bronchial muscles	Human; guinea pig; dog	H1	Arunlakshana et al, 1954; Ash and Schild, 1966; Chand and Eyre, 1975
Causes relaxation of bronchial muscles	Sheep	H2	Eyre, 1973
Increases airways resistance	Human; dog	H1	Bouhuys et al, 1960; De Koçk et al, 1966
Increases pressure in pulmonary v ascular bed	Guinea pig	H1	Turker, 1973
Decreases pressure in pulmonary vascular bed	Guinea pig	H2	Turker, 1973
Inhibits histamine release from basophils	Human	H2	Bourne et al, 1971; Lichtenstein and Gillespie, 1973 , 1975
Induces eosinophil chemotaxis in vitro	Human; guinea pig	H2	Clark et al, 1975; Turnbull and Kay, 1976; Jones and Kay, 1977
Inhibits eosinophil migration in vitro	Human	H2	Clark et al, 1975

TABLE I

Principal actions of histamine

(continued overleaf)

TABLE I (contd.)

Action	Species	Receptor Mediated	Reference
Induces release of eosinophil immobilizing factor from mononuclear cells	Guinea pig	H1 & H2	Kownatzki et al, 1977
Increases neutrophil chemokinesis and inhibits chemotaxis in vitro	Human	H2	Anderson et al, 1977
Increases cAMP in:			
Leucocytes	Human	H2	Lichtenstein and Gillespie, 1973, 1975
Lymphocytes - from spleen, lymph node and thymus	Mouse	H2	Roszkowski et al, 1977
Neutrophils	Human	H2	Busse and Sosman, 1976
Lung slices	Human	?	Beaven, 1976
Brain slices	Guinea pig	H1 & H2	Baudry et al, 1975
Inhibits antibody production in vitro	Mouse	H2?	Melmon et al, 1974
Inhibits T cell-mediated cytolysis in vitro	Mouse	H2	Henney et al, 1972; Plaut et al, 1973b
Inhibits MIF release from lymphocytes	Guinea pig	H2	Rocklin, 1976
Inhibits enzyme release from neutrophils	Human	H2	Zurier et al, 1974; Busse and Sosman, 1976

CHAPTER II - AIMS OF THE PRESENT STUDY

The respiratory tract is constantly exposed to a variety of particles including micro-organisms, allergens and potentially toxic pollutants. The airways and lung parenchyma possess several mechanisms for clearing particles such as respiratory cilia and the cough reflex. However, it is well recognized that alveolar macrophages are probably the major defence system since these cells are phagocytic, can kill various micro-organisms and, unlike other leucocytes, appear to function optimally in an aerobic environment.

It is known that activation of the "respiratory burst" in phagocytic cells, including alveolar macrophages, is achieved by a number of agents, both soluble and particulate, many of which bind to the plasma membrane (for example, particles opsonized with complement) through cell surface receptors. This suggests that there may be a close association between "membrane recognition" of certain exogenous stimuli and the enzyme which initiates the primary oxygen-consuming reaction (also thought to be membrane-bound and probably "NADPH oxidase"). The "respiratory burst" is associated with the production of a number of partial reduction products of oxygen which themselves have microbicidal capacities. These include the superoxide radical, singlet oxygen and hydrogen peroxide.

Previous studies had indicated that human and mouse leucocytes bind particles to which histamine had been coupled covalently suggesting the presence of "histamine receptors" on certain white cells (Melmon et al, 1974; Kedar and Bonavida, 1974; Saxon et al, 1977). Furthermore, these studies indicated that "histamine receptors" appeared to be particularly well expressed on mononuclear cells.

The release of histamine and other pharmacological agents from sensitized lung fragments following challenge with specific antigen is well known. The lung is a major site for histamine storage and high concentrations of histamine have been found in the sputum in pulmonary disorders associated with immediate-type hypersensitivity (Turnbull et al, 1977). For these reasons it seemed important to establish the presence of "histamine receptors" on the alveolar macrophage and to compare the expression of these receptors with other blood leucocytes.

Since, as will be shown, histamine receptors were particularly well expressed on lung macrophages, experiments were then conducted to determine whether histamine bound to particles initiates the respiratory burst in phagocytic cells in a comparable way to particles opsonized with complement.

Experiments were also carried out to determine whether histamine influenced the release of lysosomal enzymes following incubation of alveolar macrophages with serum-treated zymosan since it had been shown that, in neutrophils, histamine decreases lysosomal enzyme release during phagocytosis through a mechanism probably dependent on intracellular levels of cyclic nucleotides. At the same time, studies were also performed to see whether histamine, bound to particles, influenced lysosomal enzyme release per se, i.e. that histamine could trigger its membrane receptor for lysosomal enzyme release in the same way that complement-coated particles can initiate this secretory process.

Thus the main questions can be summarized as follows:

(1) Do alveolar macrophages have receptors for histamine and how do these compare with other cell types?

(2) Does stimulation of histamine receptors in alveolar macrophages initiate biochemical mechanisms associated with the respiratory burst?

(3) Does histamine influence the release of lysosomal enzymes by phagocytosing alveolar macrophages and what are the effects of particle-bound histamine *per se* on enzyme release?

CHAPTER III - MATERIALS AND METHODS

SECTION A - BUFFERS, REAGENTS AND PURIFICATION OF CELLS

1.0 BUFFERS AND SOLUTIONS

1.1 Dextrose-gelatin-veronal buffer ($DGVB^{2+}$) was prepared freshly each day by mixing equal volumes of isotonic veronal buffered saline (containing 0.0015 M Ca^{2+}, 0.0005 M Mg^{2+} and 0.1% gelatin veronal buffer (GVB^{2+})) and 5% dextrose in water containing equal concentrations of Ca^{2+} and Mg^{2+} ($D5W^{2+}$) as described by Nelson et al, 1966.

1.2 Gelatin veronal buffer (GVB^{2-}) was prepared as for GVB^{2+} but without Mg^{2+} and Ca^{2+}.

1.3 0.01 M EDTA GVB^{2-} - a stock solution of 0.086 M EDTA, pH 7.4, was prepared and kept at 4°C. To prepare 0.01 M EDTA GVB^{2-} this stock solution was diluted in GVB^{2-}.

1.4 Phosphate buffered saline (PBS), pH 7.2, was prepared by dissolving 8.5 g NaCl, 1.07 g Na_2HPO_4 and 0.39 g NaH_2PO_4 in 1 litre of distilled water.

1.5 Buffer standard solution (BSS), pH 7.4, was prepared by dissolving 8.0 g NaCl (0.136 M), 0.28 g KCl (0.0026 M), 1.44 g $Na_2HPO_4.2H_2O$ (0.0081 M), 0.2 g KH_2PO_4 (0.0014 M), 0.133 g $CaCl.6H_2O$ (0.0006 M) and 0.203 g $MgCl_2.6H_2O$ (0.0009 M) in 1 litre of distilled water. This solution was supplemented with 5.5 mM glucose when used as incubating medium.

1.6 Tyrode's buffer was prepared by mixing 40 ml of Tyrode's A (see below) with 1 g $NaHCO_3$, 1 g D-glucose, 1 ml 20% $CaCl_2$ and 0.4 ml 25% $MgCl_2$ in 1 litre of distilled water. The pH was adjusted to 7.4.

Tyrode's A, a stock solution containing 200 g NaCl, 5 g KCl, 1.625 g $NaH_2PO_4.2H_2O$ in 1 litre of distilled water, was prepared and stored at 4°C.

1.7 Acetate buffer 0.1 M - a stock solution was prepared by dissolving 5.79 g $CH_3COONa.3H_2O$ in distilled water; 3.25 ml of glacial acetic acid and distilled water were added up to a final volume of 1 litre. The pH was adjusted to 4.5 with 0.1 N acetic acid.

1.8 Glycine buffer 0.2 M was prepared by mixing 15.014 g glycine and 11.69 g NaCl in 1 litre of distilled water. The final pH, 10.4, was adjusted with NaOH 10 M.

1.9 Potassium phosphate (KH_2PO_4/K_2HPO_4) buffer 0.1 M was prepared by mixing 0.1 M solution of each constituent to a final pH of 7.4.

1.10 Buffered saline solution was prepared by dissolving 9 g NaCl in 1 litre of distilled water and buffered to pH 7.2-7.4 with Hepes.

1.11 Lysis solution - a hypotonic solution containing 0.155 M NH_4Cl, 0.01 M $KHCO_3$ and 0.0001 M EDTA was prepared and pH adjusted to 7.4.

2.0 CHEMICALS AND REAGENTS

Materials were obtained as follows. Histamine dihydrochloride, rabbit serum albumin, 1-ethyl-3(3-dimethyl-aminopropyl) carbodiimide HCl (ECDI), L-histidine, phenol-phthalein glucoromide, sodium pyruvate, $NADH_2$, theophylline, cytochalasin B, β-glucuronidase, bovine serum albumin and Luminol (Sigma, London); Hepes (Wellcome Laboratories, Beckenham, Kent); mepyramine maleate, phenolphthalein (May and Baker Ltd., Dagenham, Essex); chlorpheniramine (Allen and Hanburys Ltd., London); imidazoleacetic acid dihydrochloride (ImAA), 1,4-methylhistamine dihydrochloride (1,4-MeHm), N-acetylhistamine (N-AcHm) and 1-methyl-4-imidazoleacetic acid dihydrochloride (1,4-MeImAA) (Calbiochem, California, U.S.A.); Ficoll, Sephadex G-10, Sepharose 4B (Pharmacia, London); sodium diatrizoate (45% Hypaque) (Winthrop, Surbiton-on-Thames, Surrey); zymosan, chicken egg albumin (Koch Light Laboratories Ltd., Colnbrook, Bucks.); superoxide dismutase (SOD), cytochrome C (Miles Laboratories Ltd., Stoke Poges, Berks.); latex 0.81 μ (Difco Laboratories, West Molesay, Surrey); medium 199 without phenol red (Flow Laboratories Ltd., Irvine, Ayrshire); burimamide, metiamide, 4-methylhistamine dihydrochloride (4-MeHm), 2-(2-aminoethyl) thiazole dihydrochloride (2-AET) and S [3-(N,N dimethylamino propyl] isothiourea (Dimaprit) were a gift from Smith, Kline and French Laboratories Ltd., Welwyn Garden City, Herts. All other reagents were B.D.H. (Poole, Dorset) 'Analar' grade.

3.0 PREPARATION OF HISTAMINE RABBIT SERUM ALBUMIN CONJUGATE (H-RSA)

The H-RSA conjugate was prepared using the procedure described by Kedar and Bonavida (1974) with minor modifications. Briefly, 1.4 g histamine dihydrochloride, 200 mg rabbit serum albumin (RSA) and 1.2 g ECDI were incubated in 20 ml of PBS for 1 hr at room temperature with intermittent shaking. The resulting solution was dialysed at 4°C against 5 litres of PBS which was changed three times over a 48 hr period. The controls which were prepared in parallel under the same conditions were (1) RSA_{ECDI} (200 mg of RSA and 1.2 g of ECDI in 20 ml PBS) and (2) RSA_u (200 mg of RSA in 20 ml of PBS). The dialysed preparations were stored at -20°C in 1 ml aliquots and used for up to 10 weeks after preparation.

4.0 PREPARATION OF CELLS

4.1 Human alveolar macrophages were obtained from patients in which an exploratory bronchoscopy using a fiberoptic bronchoscope was performed. The lungs were washed through the bronchoscope with 50 ml sterile saline four or five times. The fluid recovered was centrifuged at 500 g for 5 min and cell pellets washed twice in BSS , and counted in a haemocytometer.

4.2 Guinea pig cells

Dunkin-Hartley strain guinea pigs weighing between 250-400 g of either sex were used throughout.

4.2.1 Alveolar macrophages were obtained by tracheobronchial lavage (Myrvik et al, 1961) with 10 ml Hepes buffered saline, pH 7.2-7.4, 10-12 times, from guinea pigs anaesthetized with intraperitoneal sodium pentobarbital (40 mg/kg). Prior to removing the lungs, the animals were exsanguinated by cutting the abdominal aorta, in order to minimize the amount of blood in the lungs. The macrophages were purified by density centrifugation on Ficoll-Hypaque ($d = 1.08\ g.ml^{-1}$) (Böyum, 1968) at 650 g for 30 min at $4^{o}C$. Cells at the interface were washed twice in PBS or BSS, and kept on ice until being rewashed in the appropriate medium and brought to the final incubation concentration used. These preparations routinely contained over 90% of alveolar macrophages with lymphocytes providing the major contamination.

4.2.2 Peritoneal macrophages and eosinophils were obtained by lavage of normal guinea pigs using 20 ml PBS containing heparin (4 units/ml). They were used either unpurified or following density centrifugation on Ficoll Hypaque as described above.

4.2.3 Peritoneal neutrophils were harvested by lavage as described above 3 hr following the intraperitoneal administration of 20 ml glycogen (1 mg/ml) in PBS.

4.2.4 Blood monocytes, neutrophils, lymphocytes and eosinophils were obtained by puncture of the abdominal aorta and collected into heparinized tubes (5 units/ml). Erythrocytes were sedimented in 3% gelatin for 15 min at room temperature. The leucocyte-rich supernatant was removed, washed three times in PBS and the cells counted to a final concentration of 5×10^6/ml in PBS.

4.2.5 Lymph node cells were collected from the paratracheal lymph nodes by gently teasing with a scalpel and filtering the resultant cell suspension through a stainless steel sieve (120 wires per inch). The cells were then washed twice in PBS and adjusted to a final concentration of 5×10^6/ml.

4.2.6 Bone marrow basophils were obtained from the humeri, tibiae and fibulae of guinea pigs given whole sheep's blood (1:1 with Alsever's solution) on 12 successive days as previously described (Dvorak et al, 1974).

If any of the preparations described above contained unacceptable numbers of contaminating erythrocytes, these were lysed by suspending the cells for 5 min at $0^{\circ}C$ in ice cold lysis solution (see 1.11), washing several times in PBS and readjusting the cell count to 5×10^6/ml. The viability of these cells was always greater than 95% as judged by trypan blue exclusion.

SECTION B - RECEPTOR ASSAYS

1.0 COMPLEMENT ROSETTE ASSAY

1.1 Sensitization with IgM (EA_M^{rab})

About 5 ml of sheep red cells (in Alsever's solution) were washed twice (at approximately 750 g for 10 min at 4°C) in 0.15 M NaCl, once in 0.01 M EDTA GVB^{2-} and the cell concentration adjusted to 1 x 10^9 cells/ml in the same solution. To these calibrated red cells the IgM fraction obtained from rabbit anti-sheep red cell serum was added in amounts causing optimal sensitization necessary for complement fixation as calculated by determining the optimal haemolysis titre. The mixture was incubated at 37°C for 30 min in a shaking water bath and at 0°C for 30 min with frequent mixing. The cells were then washed once in 0.01 M EDTA GVB^{2-} and twice in $DGVB^{2+}$. These cells were kept in $DGVB^{2+}$ at 4°C for up to one week and were washed daily with $DGVB^{2+}$.

1.2 Preparation of complement-coated sheep red blood cells (EAC)

Equal amounts of EA_M^{rab} and diluted (1/750 in $DGVB^{2+}$) fresh guinea pig serum (as a source of complement) were mixed and incubated for 30 min at 37°C in a shaking water bath. Following the incubation, the erythrocytes were spun down and adjusted to 1 x 10^8 red cells/ml in $DGVB^{2+}$.

1.3 EAC rosette formation

Equal amounts (0.1 ml) of EAC and alveolar macrophage suspensions (2 x 10^6 cells/ml in M199) were mixed, centrifuged at 500 g for 5 min and the pellets incubated at 37°C and 0°C for different lengths of time (see Results).

2.0 IMMUNOGLOBULIN ROSETTE ASSAY

2.1 Sensitization with IgG (EA_G^{rab})

Red cells were coated with the IgG fraction of rabbit anti-sheep red cell serum exactly as described for IgM except that the IgG was used at the maximum subagglutinating titre. After the final wash the sensitized cells were kept in $DGVB^{2+}$ at $4^{o}C$ at a concentration of 1×10^{8}/ml.

2.2 EA rosette formation

This procedure was the same as described previously for EAC rosette formation (see 1.3).

3.0 HISTAMINE ROSETTE ASSAY

3.1 Preparation of H-RSA-coated ox red cells

Ox red cells were washed three times in PBS and adjusted to a 50% suspension with 0.2% formal saline. After 20 min at room temperature the cells were washed three times in PBS and resuspended to the same concentration, i.e. 50%. Two and one half millilitres of various dilutions (usually 1:60) of H-RSA (or RSA_{ECDI} or RSA_u) were mixed with 0.25 ml of formalized ox cells in PBS and 20 mg of ECDI in 1 ml of PBS and incubated for 45 min at room temperature with intermittent agitation. The red cells were then washed three times in PBS and adjusted to a final concentration of 2.5×10^8 cells/ml in the same buffer.

3.2 Histamine rosette formation

Duplicate 0.1 ml volumes of leucocyte suspensions (5×10^6/ml) were mixed with an equal volume of red cells (2.5×10^8/ml), centrifuged at 500 g for 5 min at $4^\circ C$ and incubated, usually for 15 min (See Results), at $0^\circ C$ or, where necessary, at $37^\circ C$.

3.3 Inhibition of histamine rosette formation

This was studied as follows: 0.1 ml volumes of suspensions containing alveolar macrophages were centrifuged at 500 g for 5 min at $0^\circ C$ and the supernatants discarded. The test agents freshly prepared, or PBS, were added to the leucocyte pellets, resuspended and incubated for 15 min at $37^\circ C$ or $0^\circ C$. Then the red cell suspension was added, centrifuged for 5 min at 500 g and incubated for 15 min at $0^\circ C$. The samples were then treated as described above. The effect of the test agents on cell viability was assessed by trypan blue exclusion before and after treatment.

4.0 PREPARATION AND COUNTING OF ROSETTE SLIDES

4.1 Preparation

The EA, EAC and histamine rosette slides were prepared in identical fashion. After incubation the cell pellets were resuspended by gently agitating and diluted with an equal volume of 2% formal saline. Smears were made in duplicate from each suspension and dried in air (using a hairdryer).

4.2 Staining

Alveolar macrophages were stained with either May Grunwald/Giemsa or by the non-specific esterase technique described by Yam, Li and Crosby (1971) with methyl green as counterstain. All other cell types were identified by the May Grunwald/Giemsa stain.

4.3 Counting

The percentage of rosettes (those cells binding three or more erythrocytes) was counted by light microscopy in 200 random leucocytes at a magnification of x 40 (areas of cell clumping being excluded).

The percentage inhibition of rosette formation was calculated as $\left(\frac{a - b}{a}\right) \times 100$ where 'a' and 'b' represent the percentage of leucocyte rosettes in preparations pre-incubated with PBS or inhibitor, respectively.

SECTION C - SUPEROXIDE RADICAL AND CHEMILUMINESCENCE ASSAYS

1.0 ASSAY FOR SUPEROXIDE RADICAL PRODUCTION (O_2^-)

The method used was slightly modified from that described by Weening et al (1975). This is an indirect assay in which the superoxide produced by the cells is estimated by measuring the reduction of cytochrome C by the radical. The specificity of this reaction for superoxide was assessed using the enzyme, superoxide dismutase (SOD) which specifically catalyzes the conversion of O_2^- to H_2O_2. In the presence of SOD, therefore, any cytochrome C reduction is assumed to be due to reducing agents other than O_2^-.

Results were expressed as n moles of superoxide dismutase inhibitable cytochrome C reduced per 10^6 cells.

1.1 Preparation of incubation mixture

1.1.1 Cells

Alveolar macrophages separated as previously described were used at a final concentration of 2.4×10^6 cell/ml in BSS^{2+} supplemented with glucose.

1.1.2 Preparation of serum treated zymosan (STZ) and histamine treated zymosan (H-RSAZ)

Both agents were freshly prepared for each experiment by incubating 10 mg of Z with 1 ml of fresh guinea pig serum (STZ) or 1 ml of H-RSA (H-RSAZ) or control RSA_{ECDI} (RSAZ), neat or diluted in PBS for 45 min at 37°C in a shaking water bath with frequent mixing. After incubation the preparations were washed twice in buffered saline solution or PBS and readjusted to the starting volume.

1.1.3 Preparation of H-RSA bound to other particles

Sepharose 4B, Sephadex G-10 and Amberlite G-50 (10 mg)

were incubated with 1 ml of neat H-RSA washed and reconstituted in PBS under the same conditions as described for H-RSAZ.

Latex was diluted to obtain a final concentration of 10^{10} particles/ml of H-RSA and then treated as for the other particles except that the centrifugation g was at a higher speed (3000 g for 15 min) and that the washed beads were reconstituted in terms of particle numbers rather than weight.

1.1.4 Preparation of Cytochrome C

This was freshly prepared just before use and kept on ice. The concentration used in routine experiments was 12.4 mg cytochrome C/ml of buffered saline solution, although other concentrations were tested in preliminary studies (see Results).

1.1.5 Preparation of superoxide dismutase (SOD)

The enzyme was dissolved in distilled water to a concentration of 2.8 mg/ml (84 μM) and kept at -20°C. In earlier studies other concentrations similarly prepared were also tested (see Results).

1.2 Incubating conditions

The final incubation mixture in a total volume of 1 ml in 3 ml plastic tubes contained: 2×10^6 alveolar macrophages, 1 mg of STZ, H-RSAZ, RSAZ, other particles or saline when unstimulated cells were studied and 100 μM of cytochrome C. Assays were performed in triplicate: two tests in the absence of, and one in the presence of SOD (4.2 μM). All samples were kept on ice while components were added and then simultaneously transferred to a 37°C shaking water bath (180 cycles/min) to initiate the incubation. Unless stated otherwise, the incubation time was 30 min.

Reactions were stopped by placing the tubes on ice and then centrifuging for 5 min at 1000 g (except latex containing tubes which were centrifuged for 15 min at 300 g).

1.3 Histamine antagonist inhibitory assay

Mepyramine, chlorpheniramine, burimamide and metiamide were dissolved in BSS, pH 7.4, added (0.1 ml) to the cells to give final concentrations of 5×10^{-5}, 2×10^{-5} and 0.8×10^{-5} mol/l^{-1} (or BSS alone in controls) and pre-incubated for 30 min at 37°C. The stimulating agents (STZ etc.) and cytochrome C were then added and incubation performed as described above.

1.4 Determination of O_2^-

O_2^- activity was measured in the supernatant of duplicate samples as cytochrome C reduction (calculated from the decrease in absorbence at 550 nm, using an extinction coefficient of 21.1 nM^{-1} cm^{-1} on addition of excess potassium ferricyanide) using a Pye Unicam SP 1800 UV spectrophotometer. Any reduction occurring in the presence of SOD was subtracted from these values. The total amount of cytochrome C was calculated in some experiments upon the addition of sodium dithiouite.

O_2^- was expressed as nmoles of superoxide dismutase inhibitable cytochrome C reduction. When the antagonists were tested inhibition (%) was expressed as previously described (Section B, 4.3).

1.5 Determination of chemiluminescence

Chemiluminescence was measured using a slightly modified version of the technique described by Hatch et al (1968). Briefly, 0.1 ml of various dilutions of H-RSAZ or controls ($RSAZ_{ECDI}$, RSAZ, H-RSA, RSA, Z, 10^{-3} mol.l^{-1} histamine and STZ) was mixed with 0.5 ml of 0.2 nM solution of luminol in BSS, and brought to 37°C in a darkened incubator. Two million alveolar macrophages contained in 0.4 ml of BSS supplemented with 1 mg/ml (5.5 nM) of glucose were added to this mixture and counting commenced immediately. Experiments were performed in polypropylene minivials (Searle) contained in glass scintillation vials with pre-bored screwcaps (Koch-Light) which were pretreated at 37°C for 18 hr. During the preparation of incubation mixtures all manipulations were performed in the dark and at 37°C. Counts were recorded at room temperature on a Beckman LS 250 liquid scintillation counter operating in the 'out of coincidence' mode (see Henderson and Kaliner, 1978), using a minimal iso-set module adjusted to detect approximately the lower third of the tritium energy spectrum. The counting mode was set to record infinite sequential 0.2 min total count readings at a preset error of 0.2%. All assays were monitored until peak luminescence had been reached.

The incubation conditions described above were developed and established in our laboratory and were shown to be optimal for chemiluminescence by guinea pig alveolar macrophages (D.G. Jones, unpublished observations).

SECTION D - β-GLUCURONIDASE AND LACTIC DEHYDROGENASE MEASUREMENTS

1.0 β-GLUCURONIDASE AND LACTIC DEHYDROGENASE (LDH) ASSAYS

1.1 Preparation of the incubation mixture

1.1.1 Preparation of cells

Alveolar macrophages obtained as previously described (Section B, 4.2.1) were brought to a final concentration of 3.0×10^6 cells/ml in M199 without phenol red, pH 7.2-7.4.

1.1.2 Preparation of STZ and H-RSAZ

These were prepared as previously described (Section C, 1.1.2).

1.1.3 Cytochalasin B

This was prepared as a stock solution of 1 mg/ml in dimethylsulphoxide.

1.1.4 Theophylline

This was freshly prepared in M199 (without phenol red) at a concentration of 5×10^{-3} mol.l^{-1}.

1.1.5 Histamine, histamine agonists and antagonists

These were prepared as 1 ml stock solutions at a concentration of 10^{-2} mol.l^{-1} in distilled water and stored at -20°C. The stock solutions were diluted just before use in M199 (no phenol red), pH 7.4, to concentrations of 10^{-3}, 10^{-4} and 10^{-5} mol.l^{-1}.

1.1.6 H-RSA and RSA

These were diluted 1:10, 1:100 or 1:1000 in M199 (without phenol red), pH 7.4.

1.2 Incubating conditions

0.8 ml alveolar macrophage suspensions (3.0×10^6 cells/ml in M199) were preincubated with cytochalasin B (5 µl) for 5 min at 37°C. Histamine either "free" or conjugate (H-RSA), control (RSA) or histamine agonists

(0.1 ml) were added and preincubated for a further 10 min. STZ, H-RSAZ or RSAZ (0.1 ml) were then added, the mixture gassed with 95% O_2 and 5% CO_2 for 10 sec, sealed and incubated for a further 2 hr (unless stated otherwise) at 37°C in a shaking water bath and with frequent mixing. When histamine antagonists were used the drugs were added prior to histamine.

After incubation the mixtures were placed on ice, centrifuged at 1000 g for 5 min and the supernatants assayed for β-glucuronidase LDH activity and, in some experiments, total protein. The cell pellets were resuspended in 1 ml M199 and sonicated on ice (3 cycles of 4 sec each) using an MSE sonic disintegrator with amplitude setting at 14 microns, probe 3 mm.

Following centrifugation at 1000 g for 10 min β-glucuronidase, LDH and protein (in some samples) were measured in the supernatants. Total enzyme and protein activity was calculated by the addition of supernatant and pellet values. In later experiments the total LDH activity was determined in triplicate sonicates of 1 ml suspensions of untreated alveolar macrophages (2.5 x 10^6 cells/ml) prepared as described above.

1.3 Determination of β-glucuronidase

β-glucuronidase was assayed by the method of Talalay et al (1946), using phenophthalein glucuronide as a substrate. Briefly, 0.2 ml of cell supernatant, 0.6 ml of 0.1 M acetate buffer, pH 4.5, and 0.1 ml of 0.01 M phenophthalein glucuronide, pH 5.0, were incubated for 16 hr at 37°C while being shaken. The reaction was stopped by the addition of 2 ml cold glycine buffer, pH 10.4.

β-glucuronidase, with no phenolphthalein glucuronide, was used as blanks. The O.D. was read at 540 nm in a spectrophotometer (Pye Unicam SP 1800 UV). A standard curve using serial dilutions of phenolphthalein (50 to 500 μg/ml) was prepared, cold glycine buffer added and O.D. read at 540 nm.

The enzyme activity was expressed in international units (IU) defined as μmoles of phenolphthalein released per min per 10^6 cells under the incubation conditions used.

1.4 Determination of LDH

The activity of LDH was determined by measuring the initial rate of oxidation of $NADH_2$ in the presence of 0.01 M sodium pyruvate at 340 nm which under these conditions is a direct measure of the reaction of pyruvate to lactate (Kornberg and Pricer, 1951; Wroblewzki and La Due, 1955).

The enzyme activity was again expressed in IU or μmoles of $NADH_2$ oxidized per min per 10^6 cells.

1.5 Determination of protein

Protein was assayed by the Lowry method (Lowry et al, 1951) and expressed as μg/ml. A standard curve was prepared using bovine serum albumin (BSA) (0 to 500 μg/ml).

2.0 DETERMINATION OF HISTAMINE

Histamine was assayed biologically using the atropinized guinea pig ileum, suspended in oxygenated Tyrode's solution at 37°C as previously described (Brocklehurst, 1960).

SECTION E - STATISTICAL ANALYSIS

1.0 STATISTICAL ANALYSIS

All experiments were performed at least three times under the same conditions (unless stated otherwise) and the mean, standard deviations and standard errors calculated.

The degree of significance was calculated from the difference of means over the sum of standard errors (Bradford Hill, 1966).

The significance of the effects of the various inhibitory agents tested was determined by the paired t test using values from treated and untreated assayed in parallel.

CHAPTER IV - RESULTS

SECTION A - RECEPTORS ON ALVEOLAR MACROPHAGES

1.0 INTRODUCTION

The macrophage plasma membrane is essential for many of its functions, e.g. pinocytosis, phagocytosis, cell recognition and antigen binding. Some of these activities are apparently mediated through surface receptors. The exact chemical nature of receptors for opsonized particles is not known but they can be readily visualized by methods such as the "rosette technique" and immunofluorescence. For instance, receptors for IgG and/or complement (C) on many haemopoietic cells including macrophages can be visualized by exposing the cells to either antibody-coated red cells (EA) or complement-coated erythrocytes (EAC), which attach to the target cell, forming characteristic rosettes.

Receptors for the Fc portion of IgG and for C3b and C3d have been demonstrated in mononuclear cells from a variety of species. These include human monocytes and alveolar macrophages (Daughaday and Douglas, 1976; Reynolds et al, 1975), guinea pig alveolar macrophages (Rhodes, 1975) and guinea pig and mouse peritoneal macrophages (Rhodes, 1975; Griffin et al, 1975a).

In addition to receptors associated with phagocytosis, receptors for endogenous hormones including β-adrenergic catecholamines, prostaglandins and histamine have also been demonstrated in human leucocytes and mouse lymphocytes (Melmon et al, 1972).

Receptors for histamine on human leucocytes were first visualized by linking the amine via a protein carrier to Sepharose beads with subsequent incubation of this

complex with the leucocytes (Melmon et al, 1972). In this form histamine presumably adheres to its membrane receptor and cannot penetrate the cells by diffusion. It was claimed that this binding was specific for histamine since it was inhibited by several "classical" histamine antagonists (diphenhydramine, tripelennamine, antazoline and pyrilamine).

In 1974 Kedar and Bonavida described a rosette technique, using histamine linked to sheep red cells, for identifying histamine receptors. They were able to show the presence of receptors for histamine on mouse thymocytes, peritoneal exudate leucocytes, spleen cells, bone marrow, peripheral blood and lymph node cells. They also observed a decrease in the percentage of rosettes in lymphoid cells from animals immunized against tumour cells and in cells from leukaemias, mastocytoma and plasmacytoma.

Later, using the same methodology, the presence of histamine receptors in human lymphocytes was described (Saxon et al, 1977). Approximately 30% of B lymphocytes and 10% of T lymphocytes were found to form rosettes. The subpopulation of T cells bearing receptors for histamine has been associated with these cells having suppressor/cytotoxic activity (Ballet and Merle, 1976).

Histamine receptors in the small intestinal smooth muscle of cats have recently been isolated and partially characterized. Their chemical composition was protein (58.7%), phospholipid (30.8%), cholesterol (6.7%), RNA (2.8%), hexosamine (0.8%) and sialic acid (0.1%). It was concluded by the authors that the receptor-rich fraction was mainly composed of cell membrane (Uchida and Takagi, 1977).

The precise chemical relationship between histamine receptors on smooth muscle and leucocytes has yet to be established.

For the reasons stated in the introductory chapter the principal aim of this thesis is to increase our knowledge of the relationship between histamine and the alveolar macrophage. This has involved a detailed investigation on the presence and nature of receptors for histamine on lung macrophages. These results are presented in this chapter. However, at the time when these investigations commenced there was also a paucity of information on receptors for IgG and complement on alveolar macrophages. Therefore, the results of these studies (i.e. on IgG and C receptors) are also presented even though several reports on this subject have appeared in the literature since that time (Reynolds et al, 1975; Rhodes, 1975; Daughaday and Douglas, 1976).

2.0 RECEPTORS FOR IgG ON GUINEA PIG ALVEOLAR MACROPHAGES

2.1 Time course and temperature

Receptors for IgG on guinea pig alveolar macrophages were detected by the formation of rosettes using sheep red blood cells sensitized with rabbit IgG (EA_G^{rab}).

The time course and effect of temperature are shown in Fig. 3. The maximum percentage of rosettes (60.3 ± 1.4%) was achieved after 15 min incubation at 0°C, although 52.0 ± 1.2% of rosettes were formed virtually instantaneously. At 0°C a slight decrease in the number of rosettes was seen up to 60 min (53.0 ± 0.8%).

When the incubations were performed at 37°C the initial percentage of rosettes was similar but decreased after 5 min. At 60 min only 15.3 ± 3.9% of rosetting cells were seen. However, at this time almost all of the cells contained phagocytosed red cells.

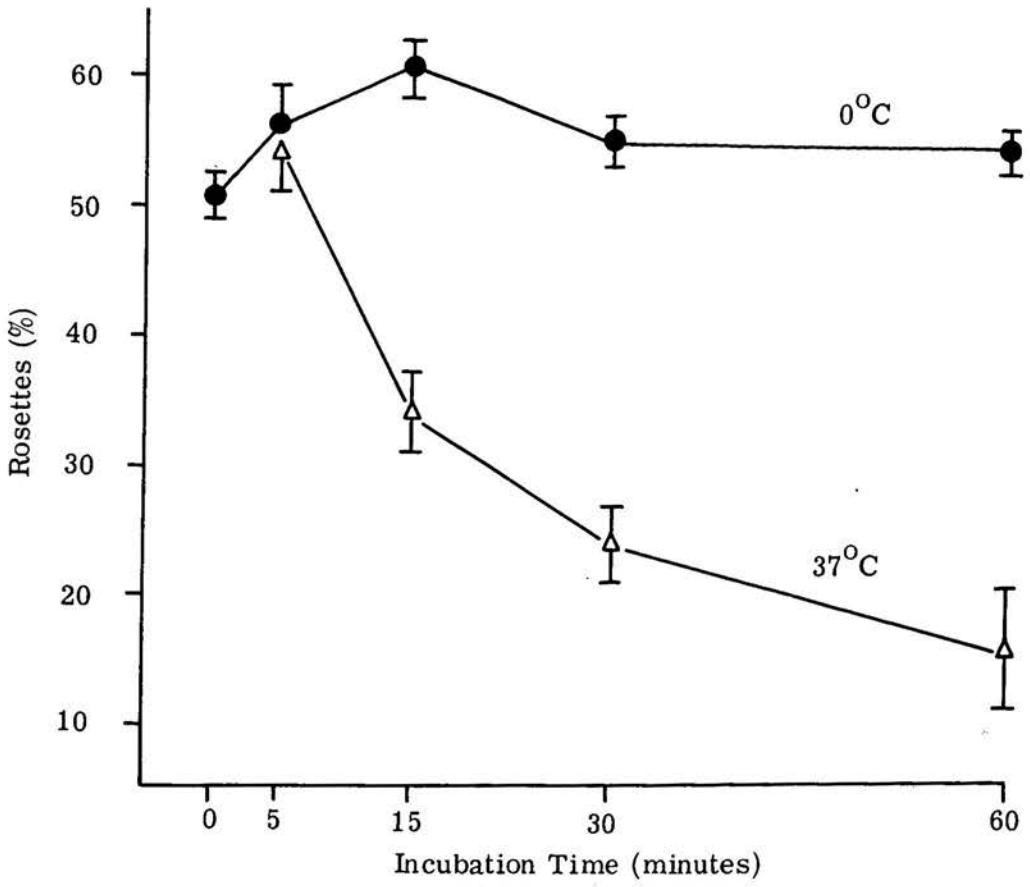


Fig. 3

The effect of variation of time and temperature of incubation on rosette formation by guinea pig alveolar macrophages with EA_G^{rab}.

The points represent the mean values (± 1 S.E.) of three experiments.

3.0 RECEPTORS FOR COMPLEMENT ON GUINEA PIG ALVEOLAR MACROPHAGES

3.1 Time course and temperature

Complement receptors were visualized on the alveolar macrophage through the formation of rosettes with sheep red blood cells sensitized with rabbit IgM (EA_M^{rab}) and coated with guinea pig complement (EAC).

The time course and effect of temperature are shown in Fig. 4. Receptors were expressed better (61.3 ± 1.4%) at 37°C and after 15 min incubation. After 15 min at 0°C the percentage of rosettes was 42.3 ± 3.3%. With an increase in the incubation time up to 60 min the number of rosettes formed at 0°C remained relatively constant (38.6 ± 4.0%), but the number of rosetting cells incubated at 37°C markedly decreased (39.6 ± 2.9%).

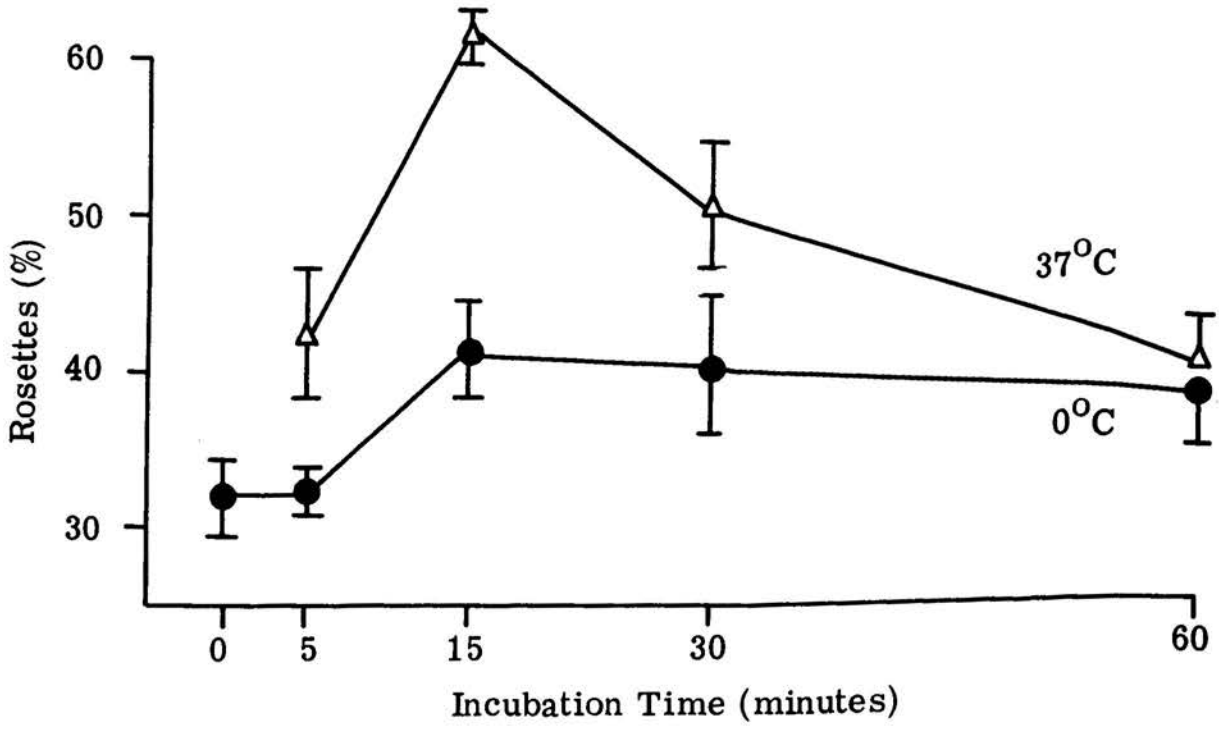


Fig. 4

The effect of variation of time and temperature of incubation on rosette formation by guinea pig alveolar macrophages with EAC.

The points represent the mean values (± 1 S.E.) of three experiments.

4.0 RECEPTORS FOR HISTAMINE ON ALVEOLAR MACROPHAGES

4.1 Determination of optimal experimental conditions

In preliminary studies the optimal conditions for detecting receptors for histamine on the alveolar macrophage were determined.

Preparation of the red cell-conjugate complex depends on several factors including (a) the species of red cell used, (b) the concentration of the coupling agent (ECDI), and (c) the concentration of histamine used in the formation of the conjugate prepared with rabbit serum albumin (H-RSA).

(a) Red cells. In initial experiments extensive red cell haemolysis was frequently a problem during the coupling of H-RSA to the erythrocytes. In an attempt to minimize this, red cells from several species including man, sheep and ox (either untreated or stabilized by formalization) were tested in the coupling reaction. Fresh (up to three days old) ox cells which were formalized immediately before use gave minimal haemolysis and, therefore, were used in all subsequent studies.

(b) Concentration of ECDI. The degree of haemolysis was also dependent on the amount of ECDI and conjugated histamine used. It was important not to use ECDI that was more than six weeks old even when stored at -20°C. When H-RSA was coupled to formalized ox red cells at a concentration of 40 mg/ml of ECDI the percentage of rosettes was greater than 80%, but there was considerable haemolysis. With H-RSA at a dilution of 1:80 the number of rosettes was high but the loss of red cells through haemolysis was excessive (Fig. 5). When the concentration of ECDI was

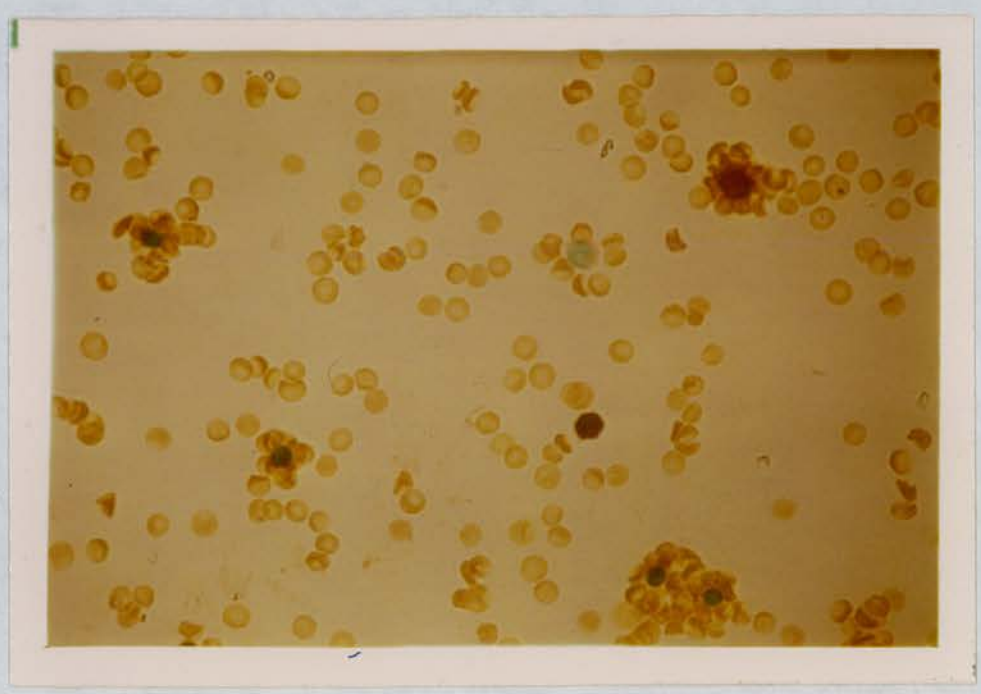


Plate 1

Rosetting assay using light microscopy.

Guinea pig mononuclear cells formed rosettes with ox red cells coated with histamine.

The slides were stained using a non-specific esterase technique. Alveolar macrophages were stained dark brown and lymphocytes counterstained green. Magnification = x 400.

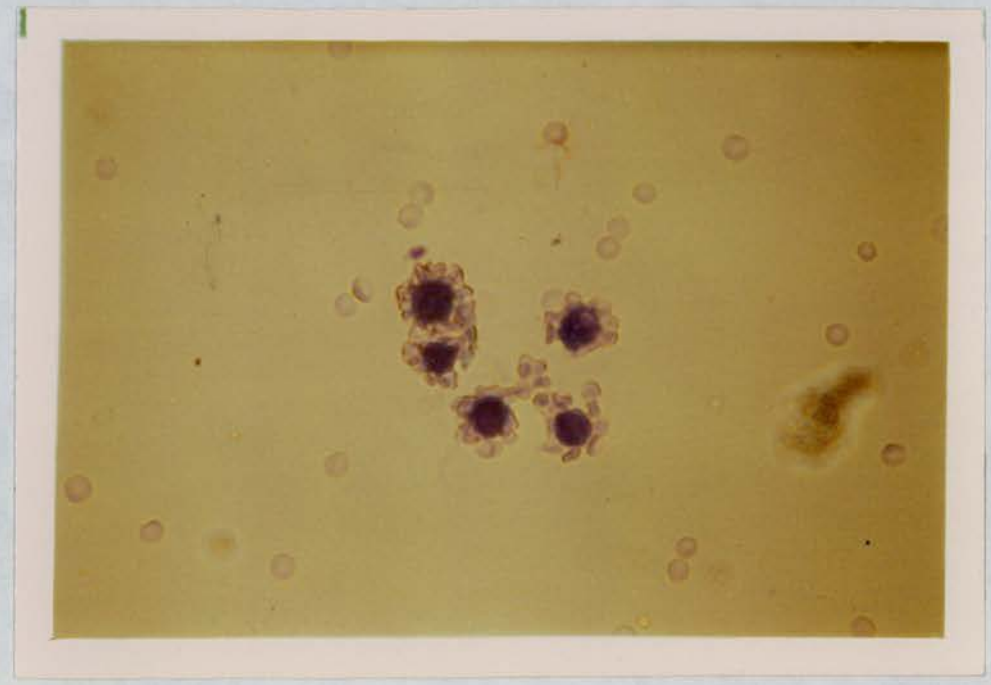


Plate 2

Rosetting assay using light microscopy.

Guinea pig mononuclear cells formed rosettes with ox red cells coated with histamine.

The slides were stained by the May Grunwald Giemsa method. Magnification = x 400.

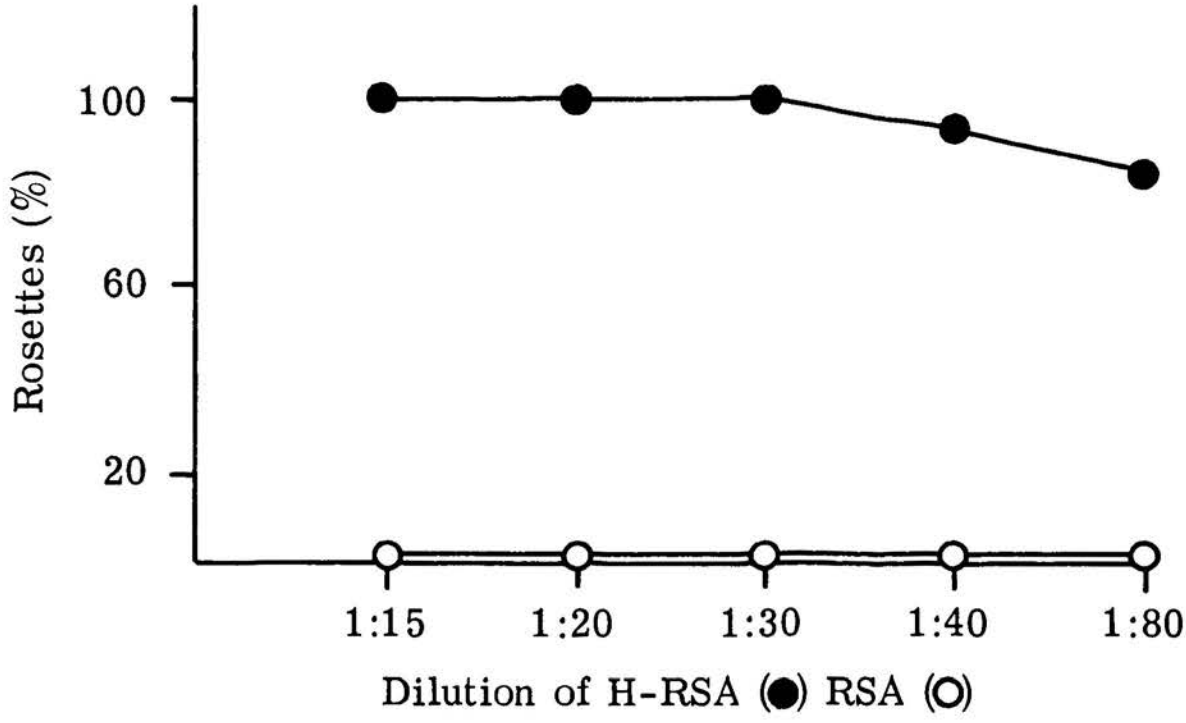


Fig. 5

The effect of dilutions of H-RSA or RSA on rosette formation by guinea pig alveolar macrophages. A concentration of ECDI of 40 mg/ml was used to couple H-RSA (●) and RSA (O) to ox red cells. The plotted points represent values obtained in one experiment.

reduced to 20 mg/ml the rosette-forming capacity of the red cell-H-RSA complex was only slightly lowered, whereas haemolysis was considerably reduced. Therefore, 20 mg/ml ECDI was routinely used for the coupling reaction.

(c) Determination of the concentration of H-RSA. The effect of varying the concentration of H-RSA coupled to formalized ox red cells using 20 mg of ECDI on the percentage of macrophage rosettes is shown in Fig. 6. The percentage of rosettes with dilutions of 1:20 to 1:80 was very similar, but considerably less rosettes were formed with 1:100 dilution.

RSA in the absence of histamine coupled to ox red cells under identical conditions did not form rosettes.

Figure 7 shows one experiment in which different dilutions of H-RSA between 1:40 and 1:100 (including those between 1:80 and 1:100) were coupled to red cells and tested for rosette formation. The percentage of rosettes decreased relatively slowly between dilutions of 1:40 and 1:80 but thereafter there was a sharp decrease.

In further experiments a concentration of 1:60 of H-RSA was used.

(d) Time course. The time course of rosette formation using ox red cells coupled to three different concentrations of H-RSA is shown in Fig. 8. Rosette formation was virtually instantaneous with only a small increase from 0 to 30 min irrespective of the dilutions. At 1:60 the number of rosettes was greater at 0.5 and 15 min when compared to the other dilutions but at 30 min were virtually identical to the 1:80 dilution.

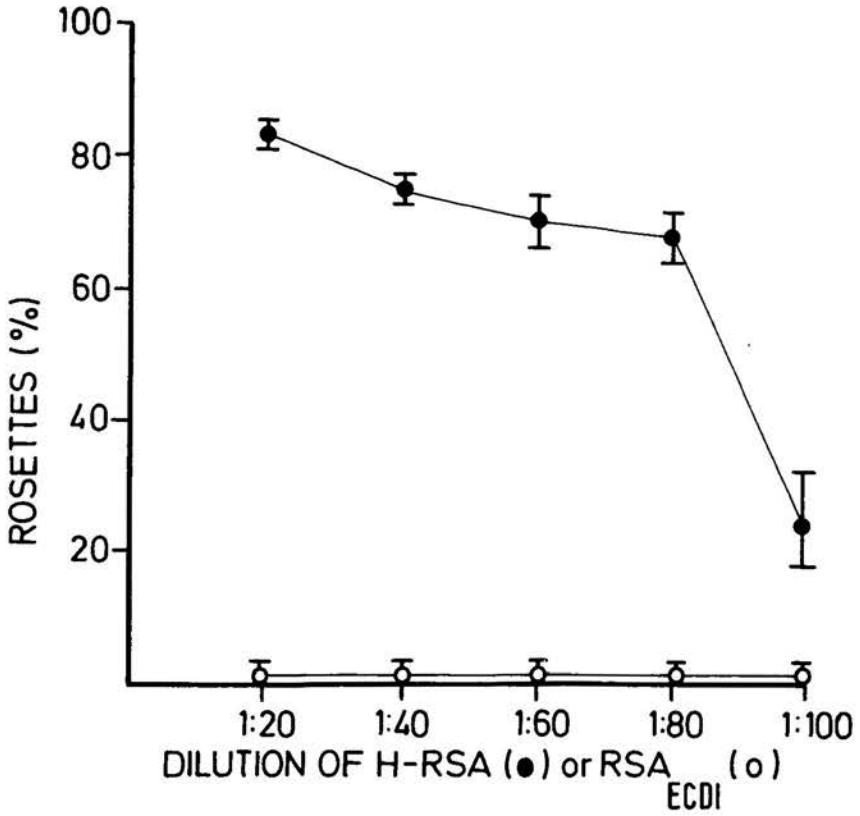


Fig. 6

The effect of dilutions of H-RSA or RSA_{ECDI} on rosette formation by guinea pig alveolar macrophages. A concentration of ECDI of 20 mg/ml was used to couple H-RSA (●) or RSA_{ECDI} (O) to ox red cells.

Each point represents the mean values (± 1 S.E.) of three experiments.

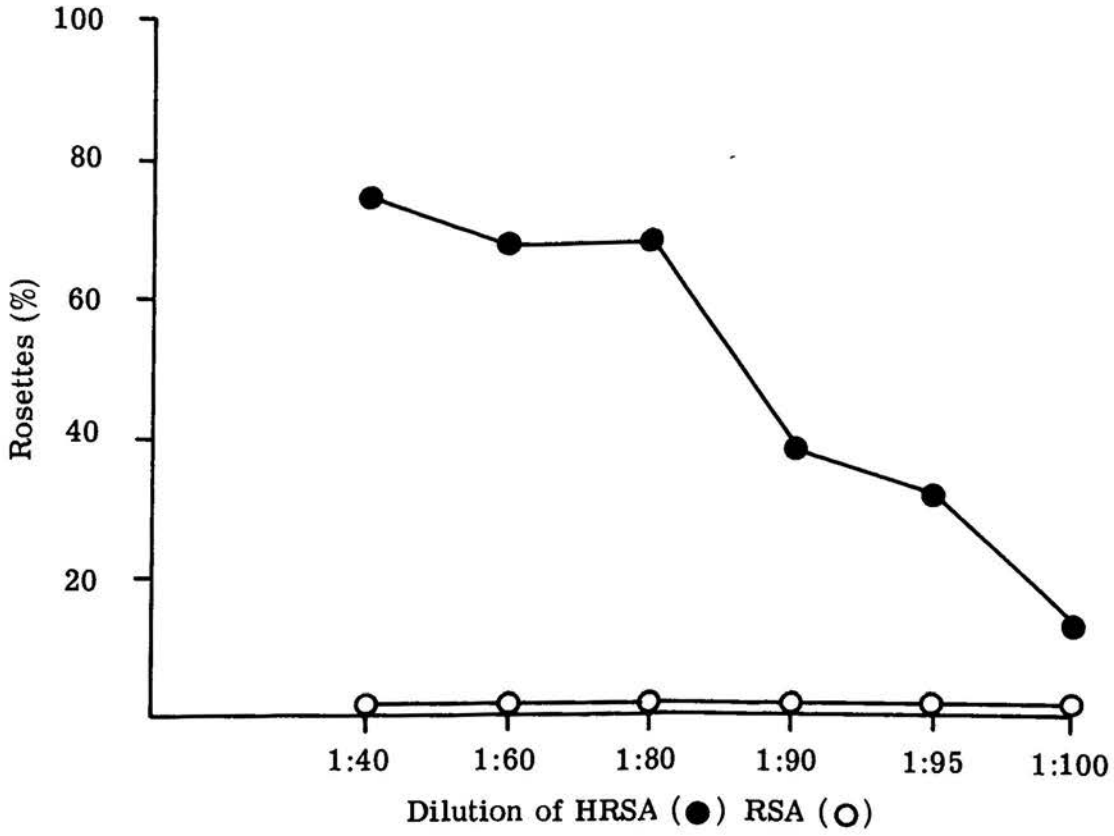


Fig. 7

The effect of dilutions of H-RSA or RSA on rosette formation by guinea pig alveolar macrophages. A concentration of ECDI of 20 mg/ml was used to couple H-RSA (●) and RSA (O) to ox red cells. The plotted points represent values obtained in one experiment.

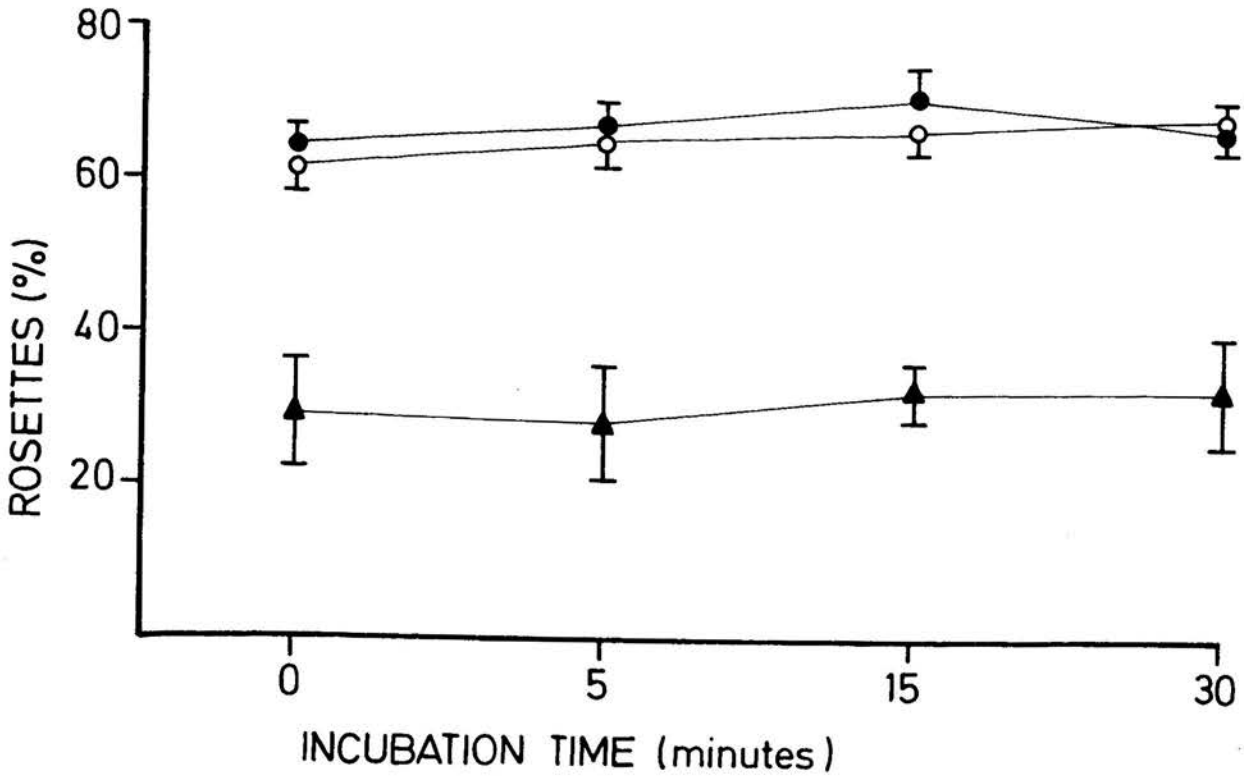


Fig. 8

The effect of incubation time on rosette formation by guinea pig alveolar macrophages with red cells coated with H-RSA dilutions of 1:60 (●), 1:80 (O) and 1:100 (▲).

Each point represents the mean values (± 1 S.E.) of three experiments.

(e) Effect of temperature. The effect of incubating alveolar macrophages with histamine conjugate bound to ox red cells at 37°C and 0°C is shown in Fig. 9. From different dilutions of conjugated histamine 1:40, 1:60, 1:80 and 1:100 were coupled to ox red cells. Virtually no difference was seen in the number of rosettes at both temperatures when dilutions of 1:40, 1:60 and 1:80 were used. There was a slight increase in the percentage of rosettes when cells were incubated with ox red cells coupled to conjugated histamine at 1:100 dilution at 37°C.

Using the optimal conditions, i.e. a 1:60 dilution of H-RSA coupled to freshly formalized ox cells with 20 mg/ml ECDI, the percentage of rosettes formed with guinea pig alveolar macrophages was always between 60 and 80%.

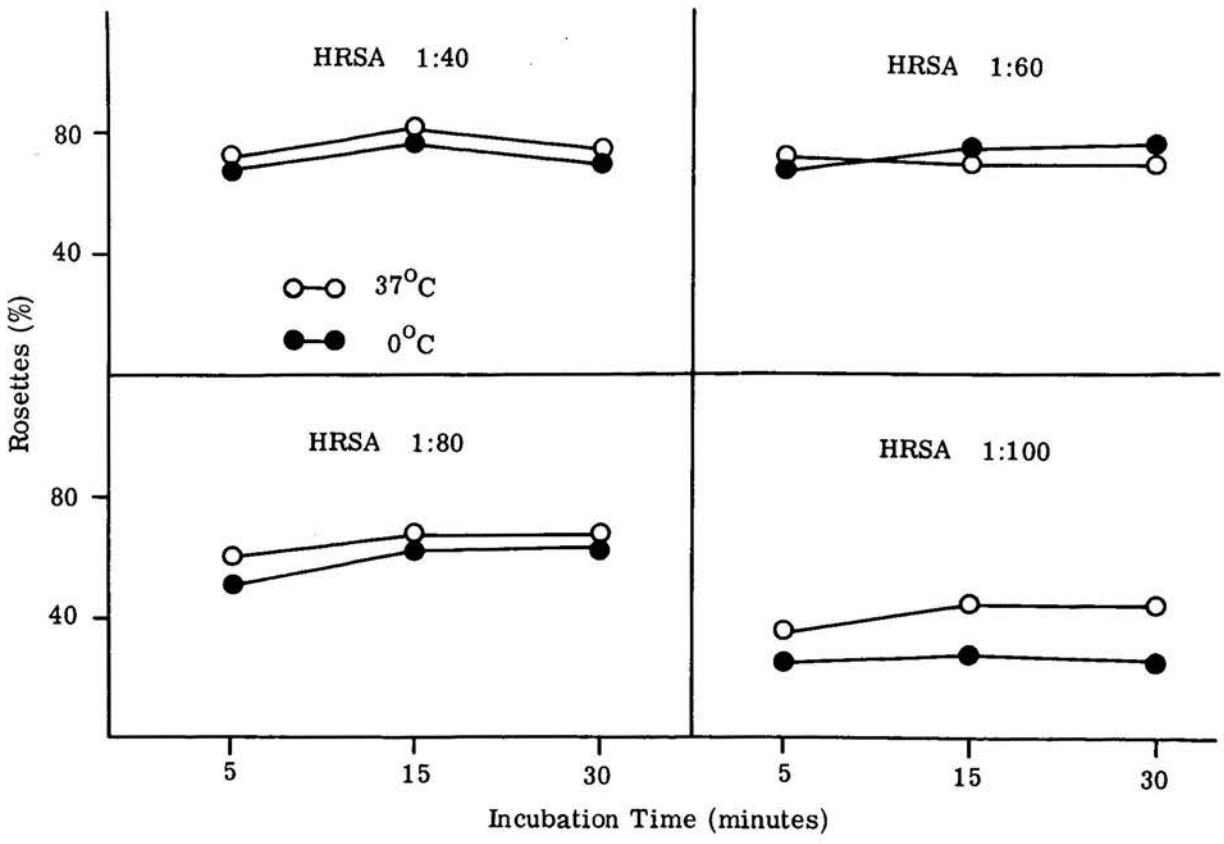


Fig. 9

The effect of incubation time and temperature on rosette formation by guinea pig alveolar macrophages with red cells coated with H-RSA dilutions 1:40, 1:60, 1:80 and 1:100.

The points represent the values obtained in one experiment.

5.0 SPECIFICITY OF HISTAMINE RECEPTORS

5.1 Inhibition of rosette formation by "free" and "conjugated" histamine

Preincubation of alveolar macrophages with "free" histamine both at 37°C or 0°C inhibited rosette formation in a dose-dependent manner from 10^{-3} to 10^{-5} $mol.l^{-1}$ (Table II). Although the inhibition was highly significant at all three doses at both temperatures ($p < 0.001$ at 0°C, 10^{-3} to 10^{-5} $mol.l^{-1}$, and at 37°C, 10^{-3} and 10^{-4} $mol.l^{-1}$, and $p < 0.05$ at 10^{-5} $mol.l^{-1}$) the maximal effect (10^{-3} $mol.l^{-1}$, 37°C) was only 48%. In contrast, preincubation of the cells with conjugated histamine completely inhibited rosette formation at 1:60 dilution whereas the equivalent amount of RSA alone or plus ECDI gave no inhibition when compared to the diluent PBS.

5.2 Inhibition of rosette formation by L-histidine and histamine metabolites

No inhibition in rosette formation was seen when alveolar macrophages were preincubated with L-histidine or the major histamine catabolites, ImAA, 1-4MeHm, N-AcHm and 1,4-MeImAA (Fig. 10). All experiments were performed in parallel with "free" histamine as a positive control.

5.3 Effect of H1- and H2-receptor antagonists

The effect of preincubation of cells with selective histamine receptor antagonists is shown in Fig. 11.

Rosette formation was inhibited by the H1 antagonists, mepyramine and chlorpheniramine, from 10^{-4} to 10^{-6} $mol.l^{-1}$ in a dose-dependent manner. Preincubation with mepyramine at 37°C and 10^{-4}, 10^{-5} and 10^{-6} $mol.l^{-1}$ gave a percentage

Pre-treatment	Number of experiments	Percentage of rosettes ± 1 S.E. (percentage inhibition)	
		0°C	37°C
PBS	12	62 ± 2	58 ± 3
Histamine: 10^{-3} mol.l^{-1}	12	35 ± 2 (44)	30 ± 2 (48)
10^{-4} mol.l^{-1}	12	42 ± 2 (32)	38 ± 2 (35)
10^{-5} mol.l^{-1}	12	49 ± 2 (21)	46 ± 3 (21)
PBS	3	66 ± 0	67 ± 3
H-RSA 1:60	3	1 ± 1 (98)	1 ± 1 (99)
RSA_{ECDI} 1:60	3	66 ± 2 (0)	69 ± 4 (0)
RSA_u 1:60	3	70 ± 1 (0)	71 ± 1 (0)

TABLE II

Inhibition of rosette formation with guinea pig alveolar macrophages by "free" histamine, H-RSA, RSA_{ECDI} and RSA_u.

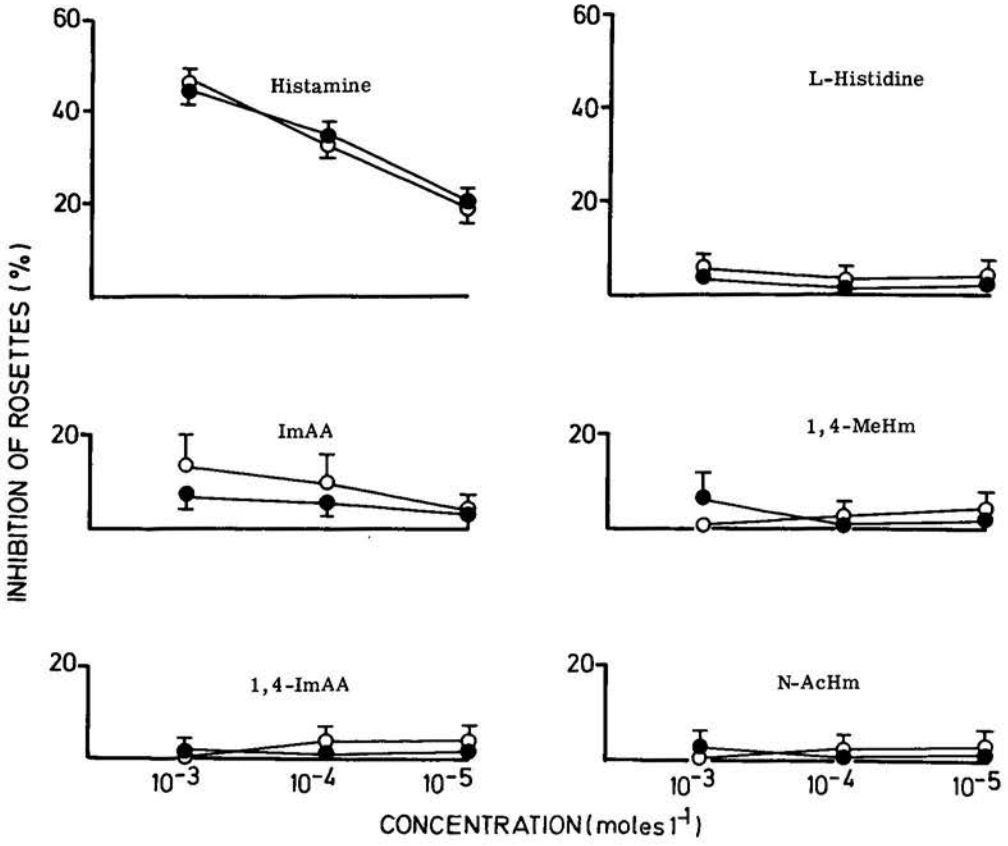


Fig. 10

Effects on rosette formation with guinea pig alveolar macrophages by "free" histamine, L-histidine, imidazole-acetic acid (ImAA), 1,4-methylhistamine (1,4-MeHm), 1,4-methylimidazoleacetic acid (1,4-ImAA), N-acetyl histamine (N-AcHm). 0°C (○) or 37°C (●)

Each point represents the mean (± 1 S.E.) of four experiments with the exception of histamine (12 experiments).

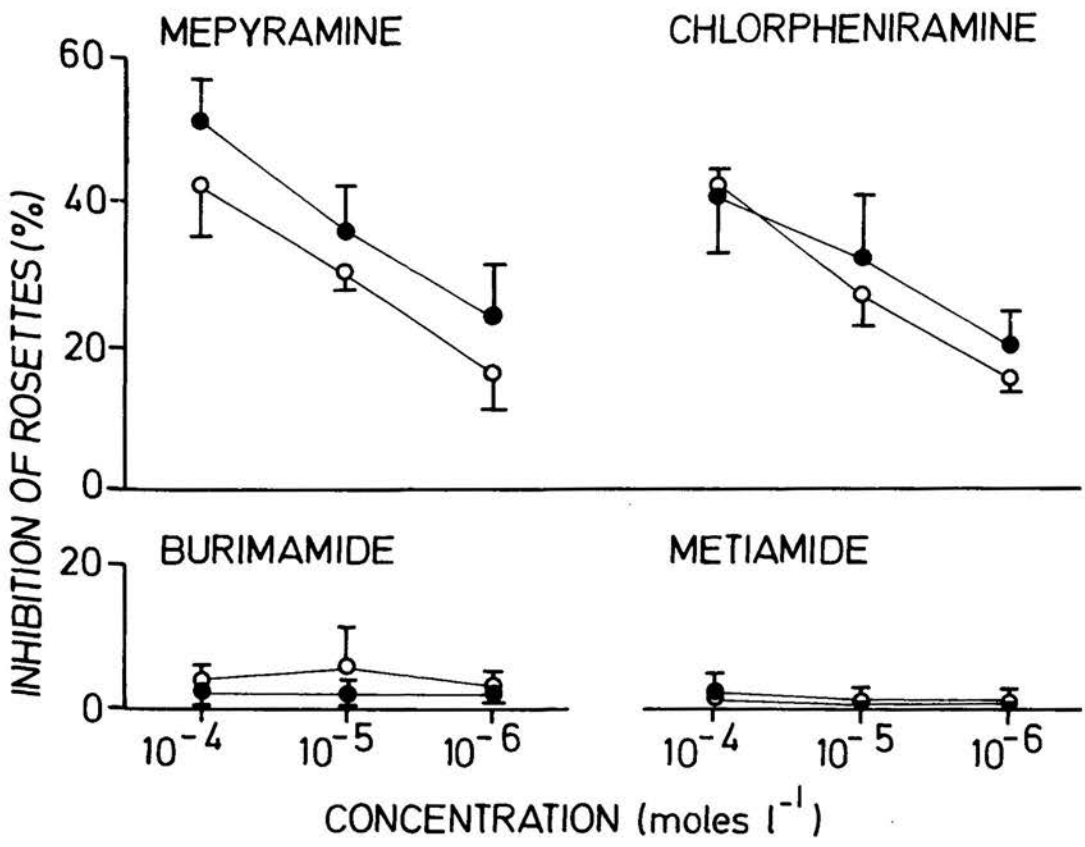


Fig. 11

Inhibition of rosette formation with guinea pig alveolar macrophages by H1- (mepyramine and chlorpheniramine) and H2- (burimamide and metiamide) receptor antagonists at 0°C (O) or 37°C (●).

Each point represents the mean (± 1 S.E.) of three experiments.

inhibition of 51.0 ± 5.9, 35.3 ± 7.6 and 24.5 ± 7.4 and a degree of significance of $p < 0.01$, < 0.1 and not significant respectively.

At 0°C and at the same doses of the antagonist a percentage inhibition of 42.3 ± 6.5, 30.3 ± 2.1 and 16.6 ± 5.2 was obtained and a degree of significance of $p < 0.001$, < 0.001 and < 0.01 respectively.

With chlorpheniramine the results were similar although less difference was observed between temperatures. At 37°C and concentrations of 10^{-4}, 10^{-5} and 10^{-6} $mol.l^{-1}$ a percentage inhibition of 40.6 ± 8.4, 32.3 ± 9.3 and 20.0 ± 4.9 respectively was seen. However it was significant only at the highest doses ($p < 0.05$).

At 0°C the inhibition of the percentage rosette formation was 41.6 ± 0.6, 27.0 ± 4.1 and 15.0 ± 0.0 at doses of 10^{-4}, 10^{-5} and 10^{-6} $mol.l^{-1}$ respectively and it was highly significant ($p < 0.001$) at all three doses.

No significant effect was seen with the H2 histamine antagonists, burimamide and metiamide.

5.4 Effect of H1- and H2-receptor agonists

The effect of preincubation of alveolar macrophages with H1- and H2- histamine receptor agonists either at 37°C or 0°C is shown in Fig. 12.

The H1-agonist, 2-AET, inhibited the rosette formation in a dose-dependent manner from 10^{-3} to 10^{-5} $mol.l^{-1}$ and it was highly significant ($p < 0.001$) at all three doses when cells were preincubated at 0°C but only with the highest doses ($p < 0.05$) when the preincubation was performed at 37°C. The maximum inhibition by 2-AET (33%) was less than that produced by a comparable dose (10^{-3} $mol.l^{-1}$) of "free"

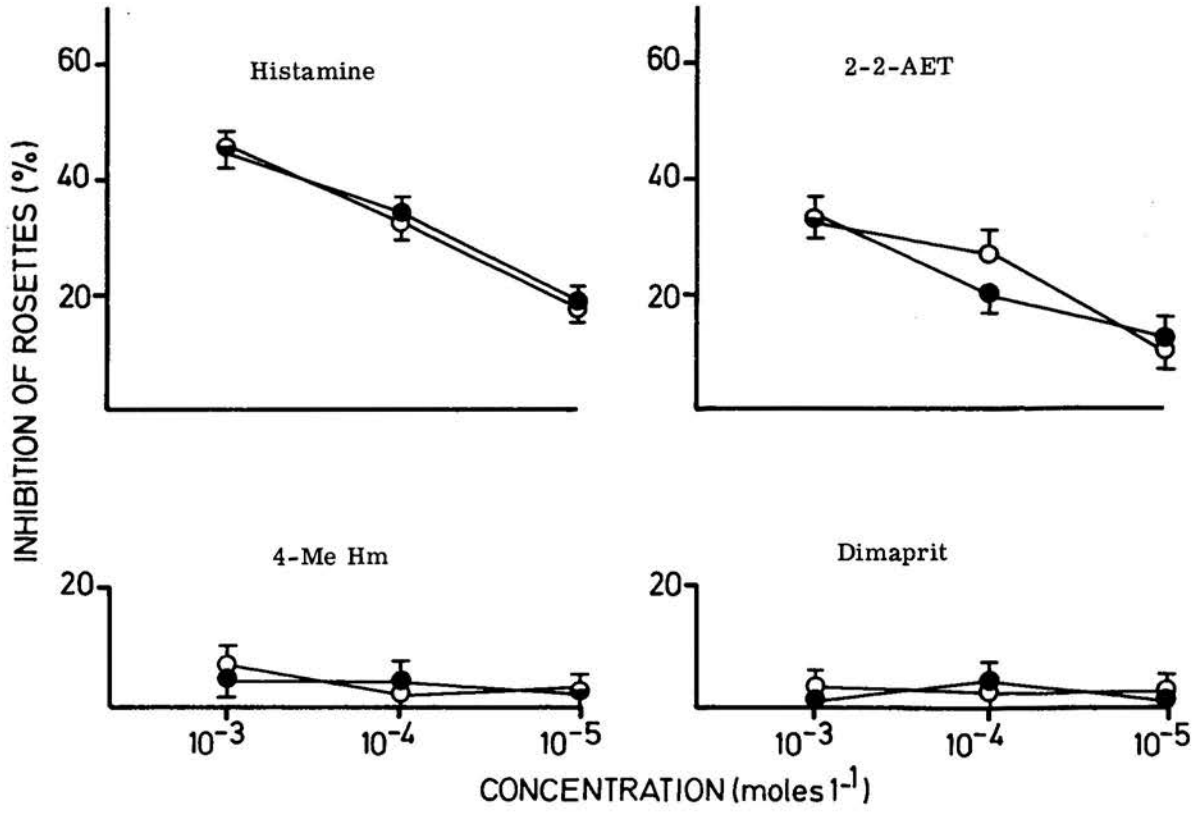


Fig. 12

Inhibition of rosette formation with guinea pig alveolar macrophages by histamine, an H1 agonist (2-2AET) and the H2 agonists, 4-MeHm and Dimaprit. Experiments were performed at 0°C (O) or 37°C (●).

Each point represents the mean (± 1 S.E.) of three experiments.

histamine (48%).

The H2 histamine agonists, 4-MeHm and Dimaprit, under the same conditions, gave no inhibition in the number of rosettes.

6.0 HISTAMINE ROSETTE FORMATION BY VARIOUS GUINEA PIG LEUCOCYTES

The percentage of histamine receptors in mononuclear phagocytes, lymphocytes, neutrophils, eosinophils and basophils is shown in Fig. 13.

In general, mononuclear cells formed more rosettes than granulocytes. Among the mononuclear cells the alveolar macrophage expressed the highest percentage of receptors. Peritoneal cells showed a great variation in the number of rosettes from one experiment to another, but generally formed higher numbers than lymphoid cells.

Rosette formation did not appear to depend on the degree of enrichment of cells, at least not by those leucocytes which were examined.

The ranges of percentage of histamine rosettes for the different cell types were alveolar macrophages 60-80%, peritoneal macrophages 14-73%, blood monocytes 14-30%, lymph node cells 27-48%, blood lymphocytes 7-24% and peritoneal and blood neutrophils 0-26% and 0-29% respectively.

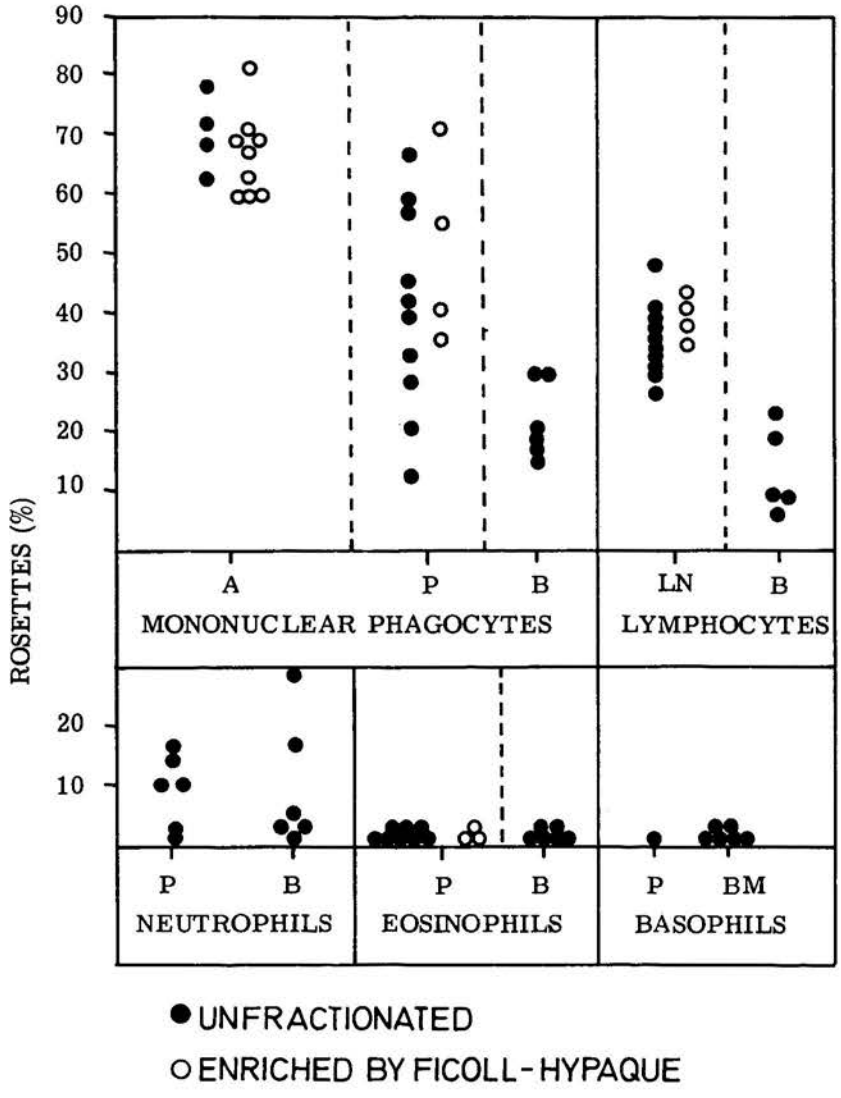


Fig. 13

Histamine rosette formation by mononuclear phagocytes, lymphocytes, neutrophils, eosinophils and basophils. In some experiments cells were unfractionated (●) or enriched (O) by centrifugation on Ficoll-Hypaque. A = alveolar; P = peritoneal; B = blood; LN = lymph node; BM = bone marrow.

Each plotted point represents one duplicated experiment.

7.0 DETECTION OF HISTAMINE RECEPTORS ON HUMAN ALVEOLAR MACROPHAGES

Alveolar macrophages were obtained from lung washings from three patients (S.D., J.T. and R.S.) on whom exploratory bronchoscopy was performed. These were tested for their capacity to form rosettes with conjugated histamine coupled to ox red cells as indicators.

The alveolar macrophages from the three patients formed rosettes (Table III). However, there was a difference in percentage. J.T. formed 30% rosettes with dilutions of 1:40 and 1:60 of conjugated histamine either at 37°C or 0°C incubation. S.D. had 60% rosetting cells with 1:60 dilution of conjugated histamine and the percentage did not vary with different times of incubation (0, 5, 15 and 30 min). The alveolar macrophages from R.S. formed less rosettes (20%) than the cells from the other two patients using the same experimental conditions.

No rosette formation was observed when alveolar macrophages were incubated with the controls (RSA_{ECDI} and RSA_u).

Patient	Dilution H-RSA	Incubation Time (min)	Temperature Incubation (^{o}C)	Rosettes (%)
J.T.	1:40	15	37^{o}	29.0
J.T.	1:40	15	0^{o}	31.7
J.T.	1:60	15	37^{o}	24.0
J.T.	1:60	15	0^{o}	31.5
S.D.	1:60	0	0^{o}	61.5
S.D.	1:60	5	0^{o}	60.0
S.D.	1:60	15	0^{o}	62.5
S.D.	1:60	30	0^{o}	64.0
R.S.	1:60	15	0^{o}	20.0

TABLE III

The effect of dilutions of H-RSA, incubation time and temperature on rosette formation by human alveolar macrophages.

8.0 SUMMARY

Various guinea pig leucocytes were tested for their capacity to bind histamine coupled as a rabbit serum albumin conjugate (H-RSA) to formalized ox red cells. The percentage of rosette-forming target cells was directly related to the concentration of erythrocyte-bound H-RSA. Under optimal experimental conditions the numbers of rosettes varied from 60 to 81% for alveolar macrophages, 14 to 73% for peritoneal macrophages, 14 to 30% for blood monocytes, 27 to 48% for lymph node cells, 7 to 24% for blood lymphocytes and 0 to 29% for peritoneal and blood neutrophils. Virtually no histamine rosettes were formed with eosinophils or basophils.

Free histamine partially inhibited rosette formation by alveolar macrophages in a dose-dependent fashion from 10^{-3} to 10^{-5} $mol.l^{-1}$, and complete inhibition was achieved by the H-RSA conjugate. In contrast, amines closely related to histamine such as L-histidine and the major histamine catabolites, ImAA, 1,4-MeHm, 1,4-MeImAA and N-AcHm, had no inhibitory effect.

The histamine H1-receptor antagonists, mepyramine and chlorpheniramine, and the H1-receptor agonist, 2-AET, all inhibited rosette formation by alveolar macrophages in a dose-dependent fashion. However, the H2-receptor antagonists, burimamide and metiamide, and the H2-receptor agonists, Dimaprit and 4-MeHm, were inactive.

These experiments suggest that (1) compared to other leucocytes, histamine receptors are particularly well expressed on the alveolar macrophage, (2) these receptors have a high degree of specificity for histamine in that other

amines, closely related chemically, did not inhibit rosette formation, and (3) the binding of histamine to the alveolar macrophage membrane is H1- and not H2-receptor dependent.

In addition, preliminary experiments on the identification of receptors for IgG and complement are reported. These confirm previous observations on the presence of these membrane markers on guinea pig alveolar macrophages.

SECTION B - THE RESPIRATORY BURST IN ALVEOLAR MACROPHAGES

1.0 INTRODUCTION

The activation of the "respiratory burst" (see Introduction) in stimulated macrophages and other phagocytes is now well recognized and has been extensively studied.

Many agents, both particulate and soluble, have been shown (1) to increase oxygen consumption, (2) to stimulate the production of superoxide radical, (3) to increase the activity of the hexose monophosphate shunt and (4) to generate increased intracellular hydrogen peroxide. The particulate agents include opsonized bacteria and zymosan (Karnovsky et al, 1975; Drath and Karnovsky, 1975; Hoidal et al, 1978a,b; Lowrie and Aber, 1977) and (at least in neutrophils) latex particles (Weening et al, 1974). Among the soluble activating agents are phorbol myristate, the active principle of croton oil (Hoidal et al, 1978a,b) and concanavalin A, a lectin obtained from jack beans (Romeo et al, 1973a). The latter is believed to bind to the cell surface by specific glycoprotein receptors (Allen et al, 1971).

Activation, which usually follows exposure to the stimulus for 30 to 60 sec (Babior, 1978; Romeo et al, 1973b), requires contact of the stimulating agent with the phagocytic surface, although the mechanism by which this contact initiates the metabolic burst is not known.

The initial phase of activation is the formation of superoxide radical by the one electron reduction of molecular oxygen (Babior et al, 1973, 1976; Johnston et al, 1975; Drath and Karnovsky, 1975).

The identity of the enzyme responsible for the primary oxygen consuming reaction of the respiratory burst has been

the subject of considerable controversy. It is generally accepted that this enzyme catalyzes the reduction of oxygen to superoxide anion through a pyridine nucleotide located in the plasma membrane (Goldstein et al, 1975a,1977; Briggs et al, 1977; Takanaka and O'Brien, 1975).

It has been suggested that the electron donor may be either NADH or NADPH. However, the present evidence indicates that in both polymorphonuclear and mononuclear cells NADPH is the primary reducing agent (Babior, 1978; Selvaraj and Sbarra, 1967; Romeo et al, 1971).

In neutrophils it has been shown that all the oxygen consumed and superoxide radical produced are derived directly from the activity of this "NADPHoxidase" and that about 50% of the superoxide radical produced is converted to hydrogen peroxide (Root and Metcalf, 1977). The rate of the superoxide radical dismutation to hydrogen peroxide is accelerated ten thousand-fold by the enzyme, superoxide dismutase (Fridovich, 1972) which would also protect the cytosol from damage by the radicals (Crapo and Tierney, 1974; Rister and Baehner, 1975).

Another potential pathway for superoxide radical utilization is the generation of singlet oxygen and hydroxyl radical in the presence of hydrogen peroxide (Johnston and Lehmeyer, 1976).

The production of singlet oxygen by phagocytic cells was initially thought to be the cause of light emission during phagocytosis (Allen et al, 1972; Johnston et al, 1976). However, other reports suggest that chemiluminescence is probably the combined result of the reaction between the

particle and some or all of the oxidizing agents generated (Cheson et al, 1976). Although chemiluminescence is a non-specific phenomenon, it has proved useful as an index of phagocytic function since respiratory burst activity is essential for light emission (Babior, 1978).

Human and guinea pig alveolar macrophages bound H-RSA red cell complex by a receptor-like mechanism (Section A). The membrane recognition mechanism might be comparable to that of complement and immunoglobulin. Stimulation of both receptors (C and Fc) initiated the respiratory burst in polymorphonuclear cells (Goldstein et al, 1975a). Therefore, it seemed important to study the metabolic response of alveolar macrophages when their histamine receptors were stimulated.

In the present study the effect of histamine on the oxidative metabolism of alveolar macrophages has been assessed by examining its effects on both superoxide radical production and the generation of chemiluminescence.

2.0 STIMULATION OF O_2^- PRODUCTION BY SERUM TREATED ZYMOSAN (STZ)

Although the assay had been previously developed in human neutrophils, it was necessary to establish the optimal baseline parameter in alveolar macrophages. These preliminary experiments are described below: (1) STZ dose response, (2) SOD concentration, (3) substrate (cytochrome C) concentration, (4) incubation medium and (5) incubation time.

2.1 STZ dose response

The guinea pig alveolar macrophage gave a dose-dependent production of superoxide radical when stimulated with opsonized zymosan (Fig. 14). The maximum response was obtained when cells (2×10^6/ml) were incubated with zymosan at a concentration of 1 mg/ml which represents approximately 10^7 particles/ml (this was equivalent to a cell/particle ratio of 1:50). At a higher concentration (2 mg/ml) the production of superoxide anion fell slightly from 39 ± 2.6 to 38.8 ± 3.6 nmoles of cytochrome C reduced. Below 1 mg/ml the amount of SOD inhibitable cytochrome C reduction was directly proportional to the amount of STZ used to stimulate the cell and even at concentrations 10 times less than optimal, superoxide levels were significantly higher ($p < 0.001$) than unstimulated cells (25.3 ± 1.6 and 10.2 ± 1.4 nmoles cytochrome C reduced, respectively).

2.2 Effect of SOD

The production of superoxide radical by alveolar macrophages was inhibited in the presence of SOD (Table IV).

To establish the amount of superoxide dismutase necessary to produce maximal inhibition, concentrations of

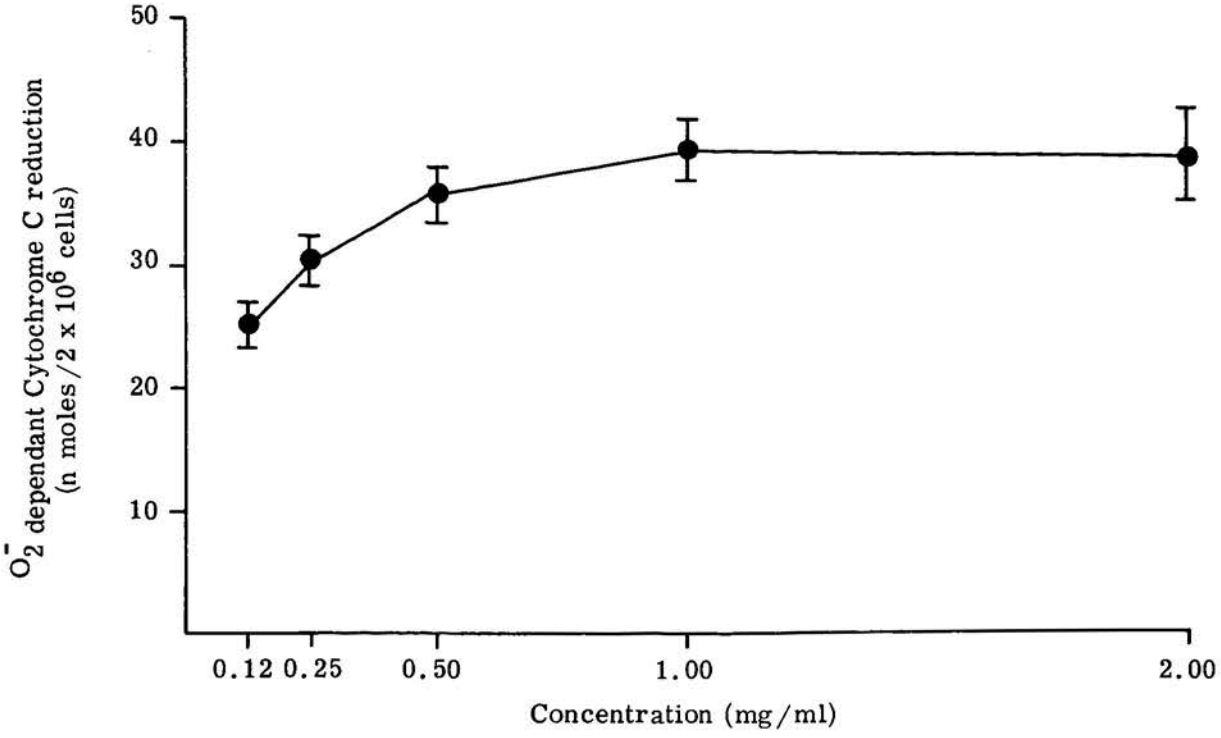


Fig. 14

The effect of guinea pig complement-coated zymosan (STZ) concentration on $O_2^{-\cdot}$ production by guinea pig alveolar macrophages.

Each point represents the mean values (± 1 S.E.) of five experiments.

Superoxide Dismutase (μM)	Cytochrome C reduction (nmoles/2 x 10^6 cells/30 min)	
	Unstimulated	Serum Treated Zymosan
No agent	10.4	45.4
0.84	5.6	10.4
1.68	1.9	8.5
2.52	1.9	4.7
3.36	0.9	0.9
4.20	0	0.9
5.04	0.9	0
5.88	0	0.9

TABLE IV

The generation of $O_2^{-\cdot}$ by unstimulated and STZ stimulated guinea pig alveolar macrophages in the presence of superoxide dismutase. The results represent the mean values for a single experiment.

the enzyme ranging from 0.84 to 5.88 μM were tested. A dose-dependent inhibition of the amount of cytochrome C reduced was observed by increasing the concentration of SOD from 0.84 to 3.30 μM, but above this no further decrease occurred. A dose of 4.2 μM of SOD was chosen for further studies. In all experiments performed using this dose the percentage of inhibition was always greater than 95%.

2.3 Concentration of cytochrome C

The assay for superoxide anion depends on the reduction of cytochrome C which must, therefore, be present in excess. In order to determine this factor, sodium dithionite, a powerful reducing agent, was added in some of the experiments. The total amount of cytochrome C used (100 μM) was always higher than that reduced in every sample.

In one experiment 2 x 10^6 cells/ml were stimulated in the presence of different concentrations of cytochrome C. A concentration of 1.2 mg/ml (100 μM) cytochrome C was sufficient for that number of cells (Table V).

2.4 Effect of incubation medium

2.4.1 Generation of superoxide radical by alveolar macrophages incubated in M199 and BSS

The capacity of alveolar macrophages to generate superoxide anion under stimulated (STZ 1 mg/ml) or unstimulated conditions was tested in two media, M199 without phenol red and the BSS previously described in the Methods. The results of the time course studies with these media are shown in Table VI.

Under resting conditions the amount of superoxide radical produced when cells were incubated in M199 was

Cytochrome C (mg/ml)	Cytochrome C reduction (nmoles/2 x 10^6 cells/30 min)	
	Unstimulated	Serum Treated Zymosan
0.6	10.4	15.6
0.9	9.9	27.9
1.2	10.4	45.4
1.5	8.5	44.5
1.8	9.9	44.5

TABLE V

The generation of $O_2^{-\cdot}$ by unstimulated and STZ stimulated guinea pig alveolar macrophages in the presence of different concentrations of cytochrome C. The results represent the mean values for a single experiment. The final concentration of STZ was 1 mg/ml.

Time (min)	Cytochrome C reduction (nmoles/2 x 10^6 cells)			
	Unstimulated		STZ	
	M199	BSS	M199	BSS
15	11.8 ± 0.3	3.4 ± 1.2	34.3 ± 0.7	31.2 ± 6.8
30	13.8 ± 1.8	5.9 ± 2.0	42.8 ± 1.8	43.9 ± 3.2
60	14.8 ± 1.8	8.2 ± 0.3	42.0 ± 1.2	43.2 ± 2.1

TABLE VI

The effect of incubation time on $O_2^{-\cdot}$ generation by guinea pig alveolar macrophages incubated in M199 and BSS. The results represent the mean values (± 1 S.E.) of three experiments.

11.8 ± 0.3, 13.8 ± 1.8 and 14.8 ± 1.8 at 15, 30 and 60 min respectively which was significantly higher ($p < 0.05$) than cells in BSS (3.4 ± 1.2, 5.9 ± 2.0 and 8.2 ± 0.3 nmoles cytochrome reduction 2 x 10^6 cells/ml at 15, 30 and 60 min respectively).

In cells stimulated with STZ the results were essentially the same irrespective of whether the cells were incubated in BSS (31.2 ± 6.8, 43.9 ± 3.2 and 43.2 ± 2.1) or M199 (34.3 ± 0.7, 42.8 ± 1.8 and 42.0 ± 1.2 nmoles cytochrome C reduced) for 15, 30 and 60 min. In both media the maximum production of superoxide radical was reached at 30 min, after which it reached a plateau.

2.4.2. Effect of adding serum and ovalbumin to the incubation medium

The superoxide dismutase inhibitable cytochrome C reduction produced by cells incubated in BSS alone or in the presence of 10% fresh guinea pig serum or 0.5% ovalbumin is shown in Fig. 15. Cells were stimulated with STZ (1 mg/ml) or latex particles (0.1 ml) and after 30 min incubation at 37°C the superoxide radical produced was measured. Both serum and ovalbumin significantly ($p < 0.001$) increased the superoxide dismutase inhibitable cytochrome C reduction in unstimulated cells (from 7.5 ± 0.5 up to 13.2 ± 0.6 and 20.8 ± 0.8 nmoles cytochrome C reduced respectively).

The amount of non-inhibitable superoxide dismutase cytochrome C reduction was increased in the presence of serum (up to 5.6 nmoles) indicating that serum contained other reducing agents. Also in the absence of cells, cytochrome C was reduced (6.3 nmoles) in the presence of serum.

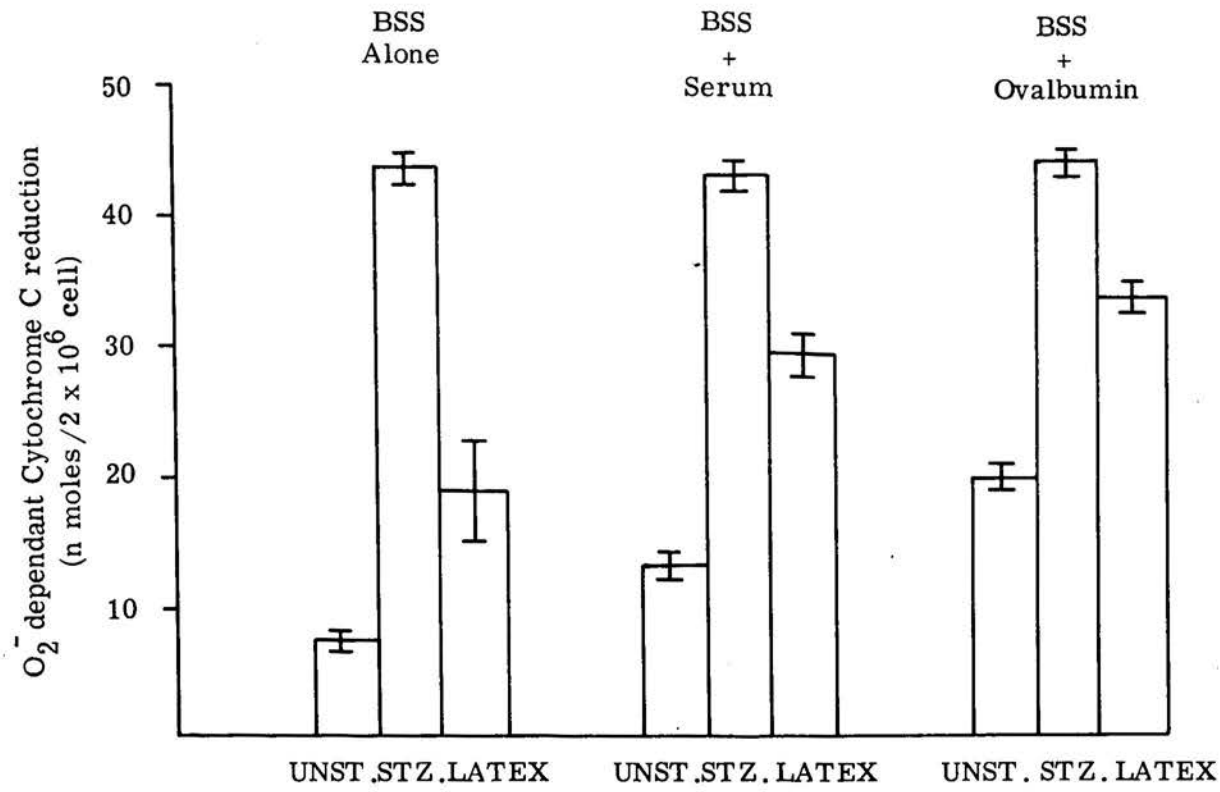


Fig. 15

The effect of serum (10%) and ovalbumin (0.5%) on $O_2^{-\cdot}$ generation by unstimulated and STZ or latex particle stimulated guinea pig alveolar macrophages.

The results represent the mean values (± 1 S.E.) of three experiments.

Cells stimulated with STZ produced similar amounts of superoxide radical whether incubated in BSS alone or supplemented with serum or ovalbumin (44.2 ± 1.0, 43.6 ± 1.6 and 44.5 ± 0.8 respectively).

Less superoxide anion was produced when cells were stimulated with latex particles (19.8 ± 4.2, 29.2 ± 2.1 and 34.0 ± 1.3) instead of STZ, under the same conditions. In the presence of serum or ovalbumin the response was higher than in its absence. However, only ovalbumin significantly ($p < 0.01$) increased the O_2^- production by latex-stimulated alveolar macrophages.

2.5 Effect of preincubation

Cells were preincubated in BSS at 37°C for 30, 120 and 180 min in a shaking water bath. Cytochrome C and either STZ or NaCl were then added to the suspension and the amount of cytochrome C reduction measured after a further 30 min incubation at 37°C. The results are shown in Fig. 16.

Preincubation decreased the capacity of unstimulated and stimulated cells to generate superoxide and the magnitude of the effect was directly related to the time used for preincubation.

Stimulated cells decreased the amount of cytochrome C reduced from 42.9 ± 3.2 with no preincubation to 38.8 ± 3.3, 36.3 ± 2.9 and 21.1 ± 2.7 nmoles at 30, 120 and 180 min respectively. However, the difference in the amount of O_2^- produced was statistically significant ($p < 0.001$) only after 180 min incubation.

In unstimulated cells the variation was from 12.3 ± 1.0 in control cells to 10.8 ± 0.4, 6.9 ± 1.0 and 5.0 ± 0.9 nmoles cytochrome C reduced (at 30, 120 and 180 min respectively).

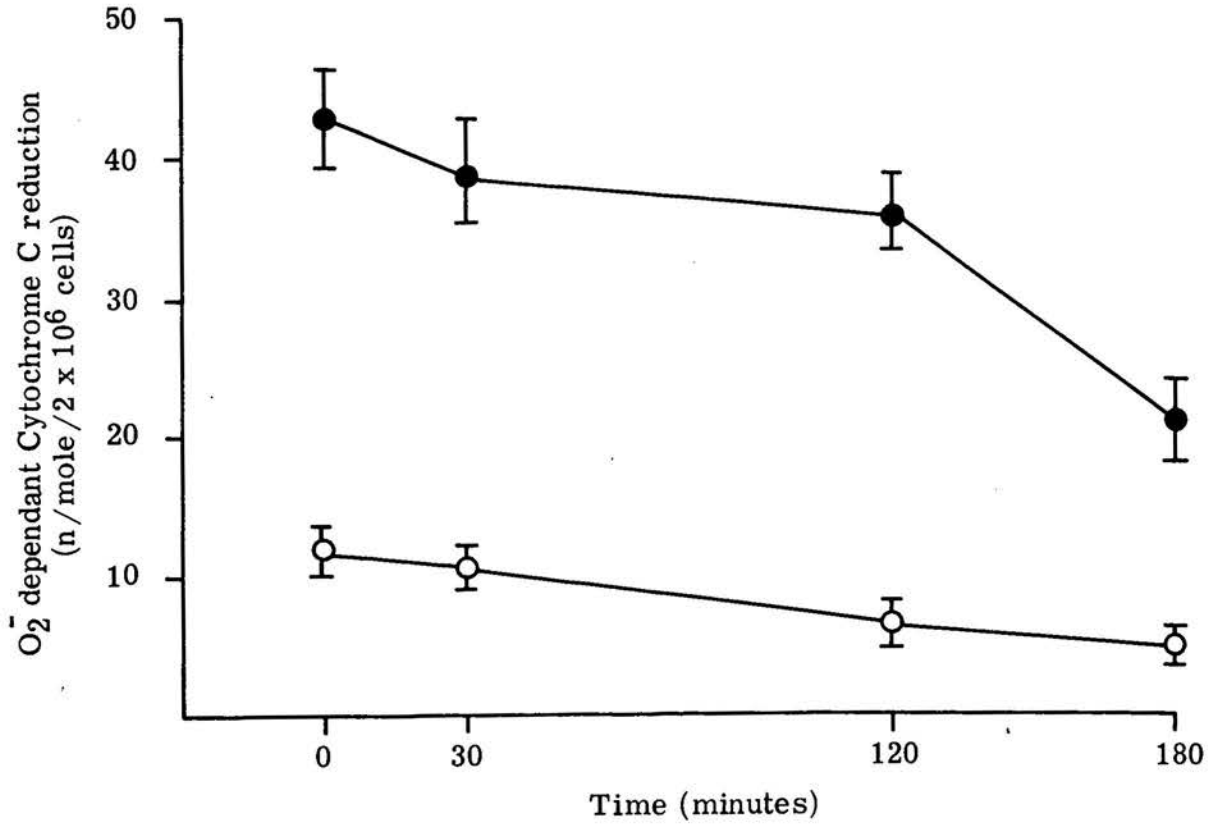


Fig. 16

The effect of preincubation time on $O_2^{-\cdot}$ generation by unstimulated (O) and STZ (●) stimulated guinea pig alveolar macrophages.

Each point represents the mean values (± 1 S.E.) of four experiments.

The reduction in O_2^- generated was highly significant after 120 min ($p < 0.01$) and 180 min ($p < 0.001$) incubation.

3.0 EFFECT OF HISTAMINE ON SUPEROXIDE RADICAL PRODUCTION

3.1 Effect of "free" histamine

These experiments were performed to determine the effects of histamine at concentrations of 10^{-4}, 10^{-5} and 10^{-6} $mol.l^{-1}$ on superoxide radical production by alveolar macrophages either unstimulated or stimulated with STZ after 30 min incubation at 37°C (Table VII). The cells and histamine were preincubated for 15 min at 37°C prior to the addition of STZ.

Resting cells in the presence of histamine produced slightly higher amounts of superoxide radical (25.0 ± 1.8, 23.9 ± 2.2 and 23.5 ± 2.5 nmoles cytochrome C reduced/ 2×10^6 cells/ml at 10^{-4}, 10^{-5} and 10^{-6} $mol.l^{-1}$ respectively) than controls without added amine (22.2 ± 2.1). This increase was dose-dependent but only statistically significant at 10^{-4} ($p < 0.05$).

No reduction of cytochrome C by histamine in the absence of cells was observed.

Stimulated cells produced less superoxide anion (43.1 ± 2.5, 44.2 ± 1.8 and 44.0 ± 2.2 nmoles cytochrome C) in the presence of histamine at 10^{-4}, 10^{-5} and 10^{-6} $mol.l^{-1}$ respectively than in its absence (47.3 ± 1.7). Also, the difference was statistically significant at 10^{-4} $mol.l^{-1}$ ($p < 0.02$).

When considered in absolute terms, histamine had little effect on O_2^- production by stimulated cells and no significant increasing effect on unstimulated values. However, when the appropriate control values (unstimulated cells) were subtracted from the stimulated values and expressed as the percentage decrease from control, histamine was found to have a dose-dependent inhibitory effect on superoxide radical.

Drug (M)	Cytochrome C Reduction (nmoles/2 x 10^6 cells/ 30 min)		Inhibition (%)
	Unstimulated	STZ Treated Cells	
No drug	22.2 ± 2.1	47.3 ± 1.7	
10^{-4}	25.0 ± 1.8	43.1 ± 2.5	30.8 ± 5.8 ($p < 0.005$)
10^{-5}	23.9 ± 2.2	44.2 ± 1.8	21.6 ± 7.6 ($p < 0.05$)
10^{-6}	23.5 ± 2.5	44.0 ± 2.2	16.7 ± 8.1 (N.S.)

TABLE VII

The effect of preincubation with "free" histamine on $O_2^{-\cdot}$ generation by guinea pig alveolar macrophages. The results represent the mean values (± 1 S.E.) of eight experiments. The inhibition of $O_2^{-\cdot}$ generation by histamine is expressed as a percentage of the control.

The percentage inhibition was statistically significant at 10^{-4} mol.l^{-1} ($p < 0.005$) and at 10^{-5} mol.l^{-1} ($p < 0.05$).

3.2 Effect of "conjugated" histamine

One experiment in which the effect of H-RSA on superoxide radical production was studied is shown in Fig. 17.

Superoxide dismutase inhibitable cytochrome C reduction was measured after 30 min incubation in the absence or presence of H-RSA at different dilutions and in the presence of RSA alone as control.

H-RSA produced a small and dose-dependent increase in the production of superoxide radical (18.1, 14.5, 11.3 and 11.3 nmoles cytochrome C reduced at 1:40, 1:60, 1:80 and 1:100 dilutions respectively) compared to control RSA at 1:40 dilution (10.7) and unstimulated cells (9.9).

H-RSA was also tested for its effects on STZ-mediated O_2^- production. For this purpose 0.12 mg/ml of STZ was used as a stimulant and H-RSA was added in 1:40 and 1:100 dilutions.

When cells were simultaneously challenged with STZ and H-RSA the effect was additive, increasing from 21.8 nmoles with STZ alone to 22.2 and 27.0 in the presence of H-RSA, 1:100 and 1:40 dilutions respectively.

3.3 Effect of "conjugated" histamine bound to zymosan (H-RSAZ) - Dose response

Different dilutions of "conjugated" histamine bound to zymosan prepared as previously described (see Methods) were tested for their capacity to generate O_2^- by the alveolar macrophage (Fig. 18).

Cells stimulated with H-RSAZ produced significantly greater ($p < 0.001$) amounts of superoxide anion in a dose response way.

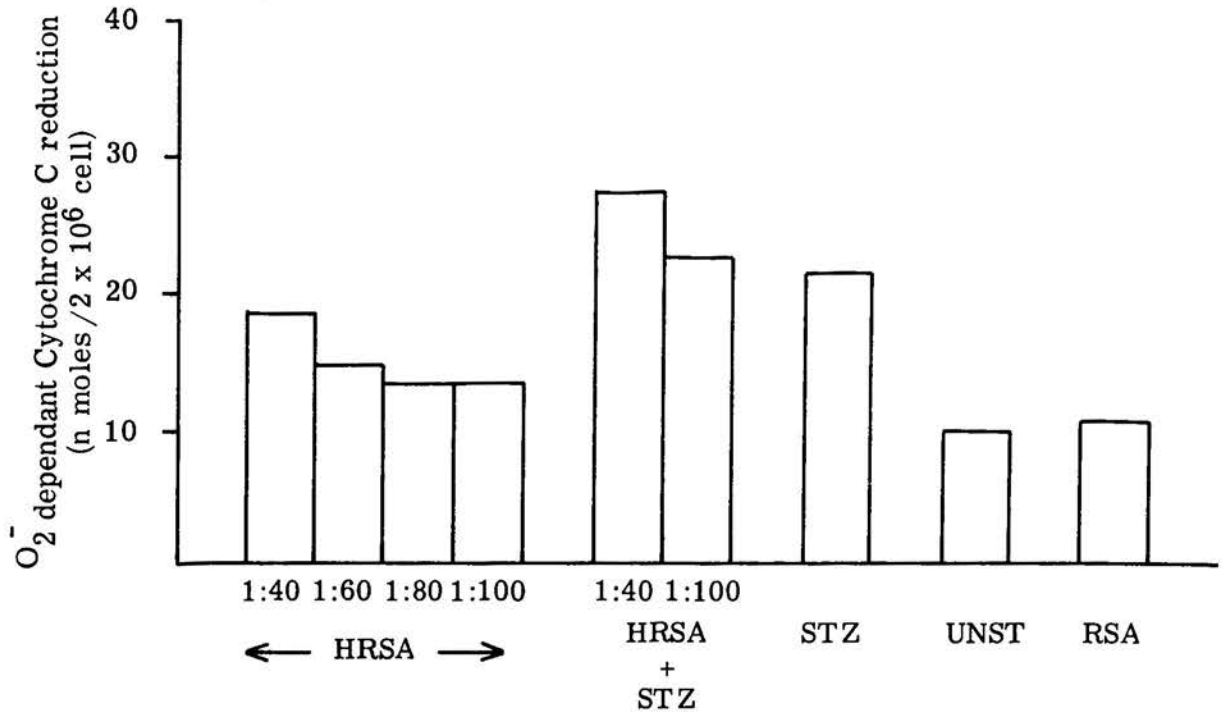


Fig. 17

The $O_2^{-\cdot}$ generation by guinea pig alveolar macrophages when treated with H-RSA, RSA and STZ. H-RSA was used at dilutions of 1:40 to 1:100, RSA at a dilution of 1:40 and STZ at a concentration of 0.12 mg/ml.

The results represent one experiment.

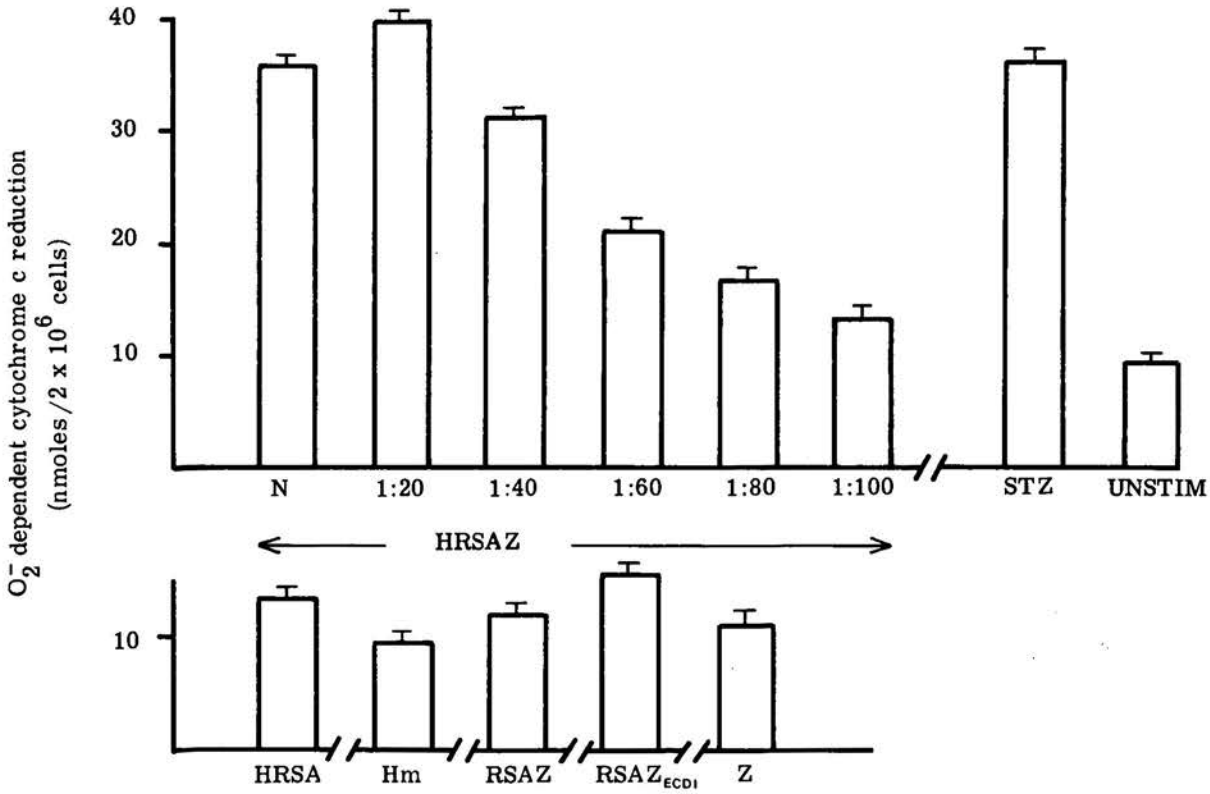


Fig. 18

The generation of $O_2^{-\cdot}$ guinea pig alveolar macrophages stimulated with H-RSAZ, STZ, H-RSA, "free" Hm, RSAZ, $RSAZ_{ECDI}$ and Z.

The results represent the mean values (± 1 S.E.) of four experiments.

When H-RSAZ neat and 1:20 and 1:40 dilutions were used the amount of O_2^- produced was 36.0 ± 1.6, 39.4 ± 2.3 and 32.3 ± 1.0 (nmoles cytochrome C reduced/2 x 10^6 cells) respectively. All these values were significantly higher ($p < 0.001$) than controls (16.8 ± 4.0, 8.0 ± 2.3, 13.8 ± 2.6, 15.9 ± 2.5 and 12.8 ± 1.2 for H-RSA, histamine, RSAZ, $RSAZ_{ECDI}$ and zymosan respectively) but not statistically different from STZ-mediated O_2^- production (36.5 ± 2.1 nmoles).

H-RSAZ at 1:60, 1:80 and 1:100 dilutions also stimulated O_2^- production by alveolar macrophages (21.8 ± 3.3, 17.5 ± 3.1 and 16.6 ± 1.2 nmoles respectively) but this was not significantly different from control values.

Although all controls used produced a higher reduction of cytochrome C than unstimulated cells, the difference was not statistically significant.

3.4 Effect of H-RSAZ - Time course

The time course of superoxide radical production by alveolar macrophages stimulated with H-RSAZ and STZ compared to controls is shown in Fig. 19.

The pattern of response was almost identical for the two stimuli tested. Superoxide radical production rapidly increased during the first 30 min, then levelled off between 30 and 60 min.

The amount of superoxide was 10.3 ± 1.5, 31.2 ± 6.8, 43.9 ± 3.2 and 43.2 ± 2.1 with STZ-treated cells and 11.5 ± 2.1, 30.7 ± 6.1, 39.1 ± 5.3 and 39.8 ± 5.6 (nmoles cytochrome C reduced) in H-RSAZ stimulated cells at 5, 15, 30 and 60 min respectively. These values were all significantly higher ($p < 0.001$) than unstimulated cells alone (1.3 ± 0.4, 3.4 ± 1.2,

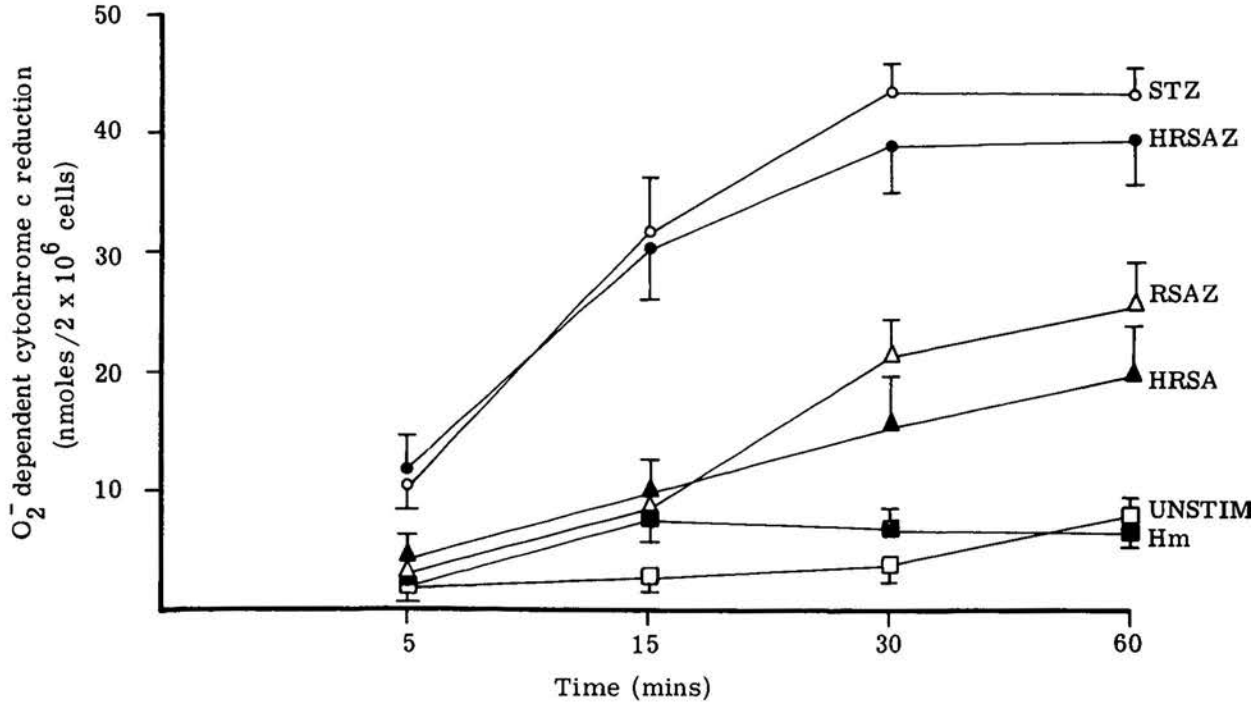


Fig. 19

The effect of incubation time on O_2^{--} generation by guinea pig alveolar macrophages activated with H-RSAZ, STZ, RSAZ, H-RSA and "free" Hm.

Each time point represents the mean (± 1 S.E.) of four experiments.

5.6 ± 2.0 and 8.2 ± 0.3 nmoles) or in the presence of histamine (1.6 ± 0.3, 9.0 ± 4.6, 8.0 ± 2.3 and 7.3 ± 1.5) measured at the appropriate times.

With RSAZ and H-RSA alone the amount of superoxide detected was less than with H-RSAZ and STZ and did not plateau even after 1 hr incubation.

$RSAZ_{ECDI}$-stimulated alveolar macrophages produced 3.9 ± 0.4, 10.2 ± 1.8, 22.8 ± 4.4 and 26.6 ± 4.0 nmoles cytochrome C at 5, 15, 30 and 60 min. The amount of O_2^- produced was significantly less ($p < 0.05$) than with H-RSAZ but statistically significantly higher ($p < 0.001$ at 5 min, $p < 0.01$ at 30 min and $p < 0.001$ at 60 min) than unstimulated cells. H-RSA-treated alveolar macrophages generated O_2^- (5.0 ± 1.7, 10.8 ± 4.0, 16.8 ± 4.3 and 19.4 ± 3.3 nmoles cytochrome C reduced at 5, 15, 30 and 60 min respectively). However, all these values were significantly less ($p < 0.05$) than H-RSAZ and not higher than controls.

3.5 Effect of H-RSAZ on superoxide radical production by human alveolar macrophages

Sufficient alveolar macrophages for metabolic studies were obtained from the lung washings of only two patients (S.D. and W.O.), who had undergone exploratory bronchoscopy.

In one patient (S.D.) cells were stimulated with H-RSAZ and STZ in parallel and the results compared to unstimulated cells and cells challenged with RSAZ as control (Table VIII). Both H-RSAZ and STZ gave an increase in O_2^- production of 23.6 and 39.8 (nmoles cytochrome C reduced) respectively from levels of 8.5 and 11.3 nmoles in unstimulated and RSAZ-treated cells. However, with STZ the amount of cytochrome C reduced was much higher than H-RSAZ-treated cells.

	Cytochrome C reduction (nmoles/2 x 10^6 cells/30 min)	
	Patients	
	S.D.	W.D.
Unstimulated	8.5	-
RSAZ	11.3	-
H-RSAZ	23.6	26.0
STZ	39.8	-

TABLE VIII

The generation of $O_2^{-\cdot}$ by human alveolar macrophages stimulated with H-RSAZ, STZ and RSAZ.

In the second patient (W.O.) only H-RSAZ was tested, in duplicate, but the results were essentially similar to those obtained with S.D. (26.0 nmoles of cytochrome C reduced/2 x 10^6 cells/ml).

3.6 Effect of histamine antagonists

Several specific histamine receptor antagonists were examined for their effect on H-RSAZ-mediated superoxide production (Fig. 20).

The H1 antagonists, chlorpheniramine and mepyramine, gave a linear dose-dependent inhibition of 22.3 ± 3.8, 13.8 ± 3.6, 10.7 ± 4.4 and 24.4 ± 5.0, 19.0 ± 4.8, 12.8 ± 3.3 respectively at 5 x 10^{-5}, 2 x 10^{-5} and 0.8 x 10^{-5} mol.l^{-1}. The inhibition was highly significant ($p < 0.005$) with both drugs at all concentrations.

The H2 antagonists, burimamide and metiamide, had virtually no effect on superoxide radical.

None of the agents tested affected the production of superoxide anion by unstimulated cells. Also, the agents were not cytotoxic, as judged by trypan blue dye exclusion of cells after incubation.

In order to determine whether the inhibition of superoxide radical generation was a specific blocking effect of the histamine receptor and not a non-specific interaction with the cell membrane, cells were preincubated with the H1 histamine antagonists, mepyramine and chlorpheniramine, at 5 x 10^{-5} mol.l^{-1} and stimulated with H-RSAZ and STZ (Fig. 21). Cells stimulated with H-RSAZ inhibited superoxide radical production by 23.6 ± 3.1 % and 26.1 ± 1.5% with chlorpheniramine and mepyramine respectively. The inhibition was statistically

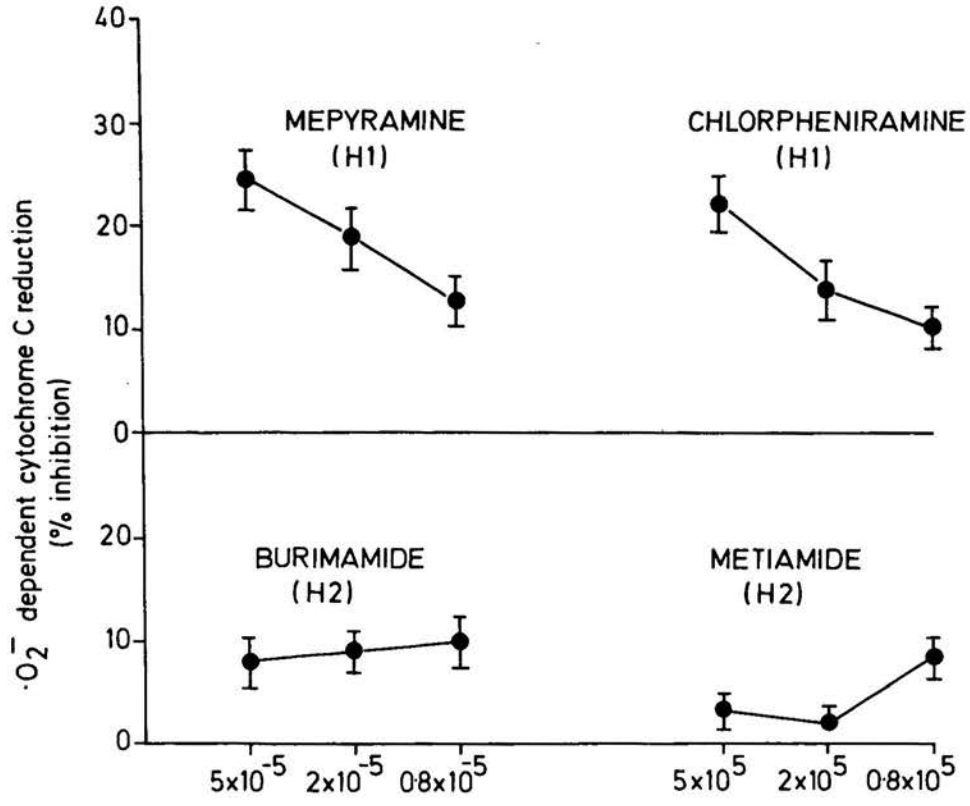


Fig. 20

Inhibition of $O_2^{-\cdot}$ generation with H-RSAZ stimulated guinea pig alveolar macrophages by H1- (mepyramine and chlorpheniramine) and H2- (burimamide and metiamide) receptor antagonists.

Each point represents the mean (± 1 S.E.) of six experiments.

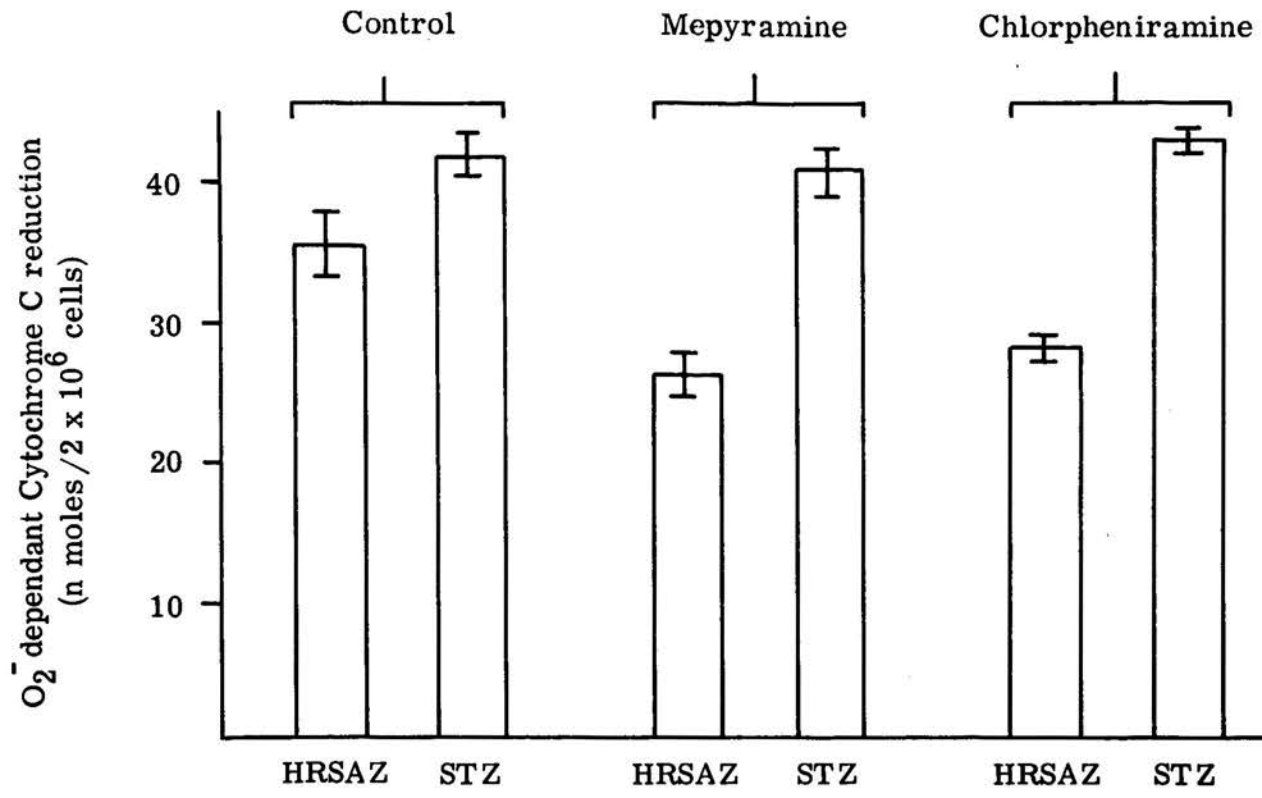


Fig. 21

The generation of $O_2^{-\cdot}$ by H-RSAZ and STZ stimulated guinea pig alveolar macrophages incubated with H1 (mepyramine and chlorpheniramine) histamine antagonists at 5 x 10^{-5} mol.l^{-1}.

The bars represent the mean values (± 1 S.E.) of two experiments.

significant with both drugs ($p < 0.02$). When the cells were stimulated with STZ the amount of O_2^- generated was the same in the presence and absence of the H1 antagonists.

3.7 Conjugated histamine bound to other particles

Cells stimulated with conjugated histamine bound to carriers other than zymosan also generated superoxide radical (Table IX).

The particles used were of several types and sizes and included (1) Sephadex G-10, a cross-linked dextrose gel (40-120 μM), (2) Sepharose 4B, a bead-formed agarose gel (60-140 μM), (3) Amberlite CG-50, a cross-linked polystyrene polymer (200-400 μM), (4) latex (polystyrene) beads (1.1 μM).

These particles by themselves or following the addition of rabbit serum albumin alone did not produce an appreciable amount of superoxide anion.

The maximum superoxide dismutase inhibitable cytochrome C reduction was achieved with H-RSAZ (39.2) followed by H-RSA-coated Sephadex G-10 (34.1 ± 0.9), Sepharose 4B (29.3 ± 2.0), Amberlite CG-50 (29.3 ± 2.2) and latex (23.6 ± 0.7) (nmoles cytochrome C reduced/2 x 10^6 cells).

All H-RSA-coated particles generated significantly higher ($p < 0.05$) amounts of superoxide radical than particles alone or coated with RSA.

Particle	Diameter (μM)	Cytochrome C reduction (nmoles/2 x 10^6 cells/30 min) HRSA	RSA	Neat
Latex	1.1	24.5 ± 0.4	13.2 ± 2.5	15.8 ± 0.9
Zymosan	<10	38.0 ± 0.8	12.2 ± 1.2	11.1 ± 0.8
Sephadex G-10	40-120	32.6 ± 0.9	11.1 ± 0.8	9.2 ± 0.6
Sepharose 4B	60-140	27.4 ± 1.1	11.6 ± 0.6	10.3 ± 1.1
Amberlite CG-50	200-400	26.1 ± 1.2	11.9 ± 1.4	9.8 ± 0.2

TABLE IX

The generation of $O_2^{-\cdot}$ by guinea pig alveolar macrophages stimulated with coated (H-RSA and RSA) and uncoated particles. The results represent the mean values (± 1 S.E.) of three experiments.

4.0 CHEMILUMINESCENCE PRODUCED BY ALVEOLAR MACROPHAGES STIMULATED WITH HISTAMINE AND SERUM TREATED ZYMOSAN

4.1 Dose response and time course

Both H-RSAZ and STZ produced light emission from stimulated alveolar macrophages. Conjugated histamine, at different dilutions, bound to a fixed amount of zymosan (10 mg/ml) gave a dose-dependent chemiluminescence which was maximal at a dilution of 1:10 (Fig. 22).

The peak of light emission was reached at 6-10 min after stimulation with all doses of the agents studied.

No chemiluminescence was observed with the controls, RSAZ, H-RSA and histamine at 10^{-4} $mol.l^{-1}$.

Superoxide dismutase (4.2 μM) completely abolished the light emission by cells stimulated either by H-RSAZ or STZ.

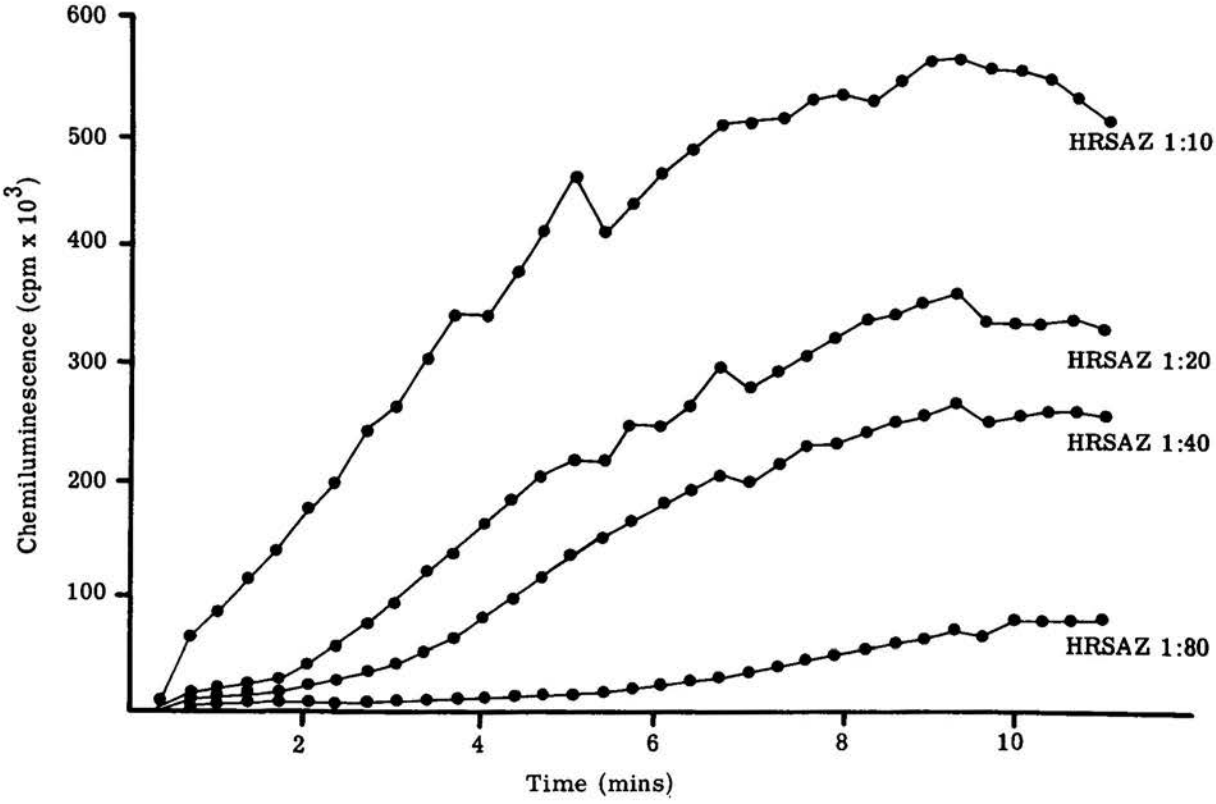


Fig. 22

The effect of incubation time on chemiluminescence by guinea pig alveolar macrophages activated with different dilutions of H-RSAZ. The results are expressed in counts per minute.

The figure is representative of six experiments.

5.0 SUMMARY

Activation of the respiratory burst in phagocytic cells is achieved by a number of agents, both soluble and particulate, many of which bind to the plasma membrane through cell surface receptors. This suggests that there may be a close association between "membrane recognition" of certain exogenous stimuli and the enzyme which initiates the primary oxygen-consuming reaction (also thought to be membrane-bound and probably "NADPH oxidase"). Since alveolar macrophages bind red cells coated with histamine, as well as complement and IgG-coated erythrocytes, experiments were performed to determine whether histamine-coated particles generate activities associated with the respiratory burst. It was shown that histamine-coated particles generated superoxide (O_2^-) and chemiluminescence in alveolar macrophages. The binding of histamine was achieved by first coupling the amine to rabbit serum albumin (RSA) and then allowing this H-RSA conjugate to absorb on to the particle. The majority of experiments were performed with zymosan although several other particles which absorbed the coupled histamine also generated O_2^-. The amount of O_2^--dependent cytochrome C reduction achieved with the H-RSA conjugate was comparable to that obtained with serum treated zymosan (STZ). Superoxide production was also dependent on the time of incubation and was attributable to alveolar macrophages and not to contaminating leucocytes.

Chemiluminescence, which also accompanies the respiratory burst of phagocytic cells, was initiated by histamine bound to zymosan. This effect was also time- and dose-dependent.

Chemiluminescence induced by H-RSAZ or STZ was completely inhibited by superoxide dismutase indicating that light emission by these cells was closely associated with O_2^- production. These results suggest that there may be a direct relationship between histamine and the lung macrophage and that histamine may be concerned with the regulation of oxygen-dependent events in the alveolar macrophage.

SECTION C - THE EFFECT OF HISTAMINE ON THE RELEASE OF LYSOSOMAL ENZYMES BY ALVEOLAR MACROPHAGES

1.0 INTRODUCTION

The discharge of enzymes into phagosomes is an important event in physiological and pathological mechanisms of microbial killing by phagocytic cells. Under certain circumstances, lysosomal products can be released into the extracellular fluid and in this may contribute to tissue damage in various acute and chronic inflammatory states.

Two principal mechanisms by which lysosomal enzymes are detected in the incubation medium without loss of cell viability have been studied *in vitro*.

(i) During phagocytosis of a variety of particles, e.g. opsonized zymosan, microorganisms, immune complexes etc. the phagolysosome formed can open to the exterior, or degranulation into the phagocytic vacuole may occur before the closure to a complete vacuole (Weissmann *et al*, 1971a; McArthur *et al*, 1976; Cardella *et al*, 1974). This process has been called "regurgitation during feeding" (Weissmann *et al*, 1971b).

(ii) The second mechanism has been named "reverse endocytosis" (Weissmann *et al*, 1972) or "frustrated phagocytosis" (Henson, 1971) in which phagocytic cells exposed to non-phagocytozable particles, e.g. a solid surface coated with immune complexes (Henson, 1971) or cells treated with cytochalasin B, and exposed to opsonized zymosan, fuse their lysosomes with the plasma membrane and release their content into the area of the particle contact (Weissmann *et al*, 1973).

It has been suggested that cyclic nucleotides might play a role in controlling lysosomal enzyme release in human neutrophils (Weissmann *et al*, 1971b; Ignarro *et al*, 1974a,b).

Cyclic GMP and cholinergic agents enhanced enzyme release (Ignarro and George, 1974) whereas cyclic AMP and agents which increase the levels of this nucleotide such as β-adrenergic agonists, prostaglandins E_1, E_2, and A_2, histamine and others, inhibited degranulation (Goldstein et al, 1973; Ignarro et al, 1974a,b; Ignarro, 1974; Zurier et al, 1974). Histamine-induced inhibition of enzyme release was apparently blocked by metiamide (an H2-receptor-dependent antagonist) (Busse and Sosman, 1976).

In macrophages the role of cyclic nucleotides in modulating enzyme release is unclear, although mouse peritoneal macrophages, like human neutrophils, were similarly affected by agents which influence the cyclic AMP/GMP balance (Weissmann, 1971b). In guinea pig alveolar macrophages, cyclic nucleotides, adrenergic and cholinergic agents had no effect on enzyme release (Ackerman and Beebe, 1975).

Following the observation that alveolar macrophages had well expressed histamine receptors (Section A) and that histamine conjugated to a protein bound to particles was able to initiate the metabolic burst in these cells (Section B), it seemed important to study the effect of histamine on enzyme release by the guinea pig alveolar macrophage.

2.0 DETECTION OF ENZYME RELEASE BY STIMULATED ALVEOLAR MACROPHAGES

2.1 Effect of histamine

The release of β-glucuronidase and lactic dehydrogenase by cytochalasin B-treated alveolar macrophages stimulated with STZ in the presence or absence of histamine (10^{-4} $mol.l^{-1}$) is shown in Fig. 23.

STZ significantly increased both the amount of β-glucuronidase released into the incubation medium ($p < 0.001$) and the total amount of this enzyme ($p < 0.01$), i.e. residual and released enzyme. Non-opsonized zymosan (Z) had no effect on these variables.

Histamine significantly increased ($p < 0.01$) the amount of β-glucuronidase released from cells stimulated with STZ, but not in unstimulated cells. However, the total amount of enzyme was almost identical in STZ-treated cells in the presence or absence of histamine (10^{-4} $mol.l^{-1}$).

The release of LDH during the time of incubation was insignificant and no differences were found irrespective of whether cells were unstimulated or stimulated with STZ, or whether they were incubated in the presence or absence of histamine. Also, the total amount of LDH was unaffected by these treatments.

The cell protein was also measured in both the supernatant and the pellet. A relatively constant amount was found in those experiments in which this was measured and no differences were found under the various experimental conditions studied.

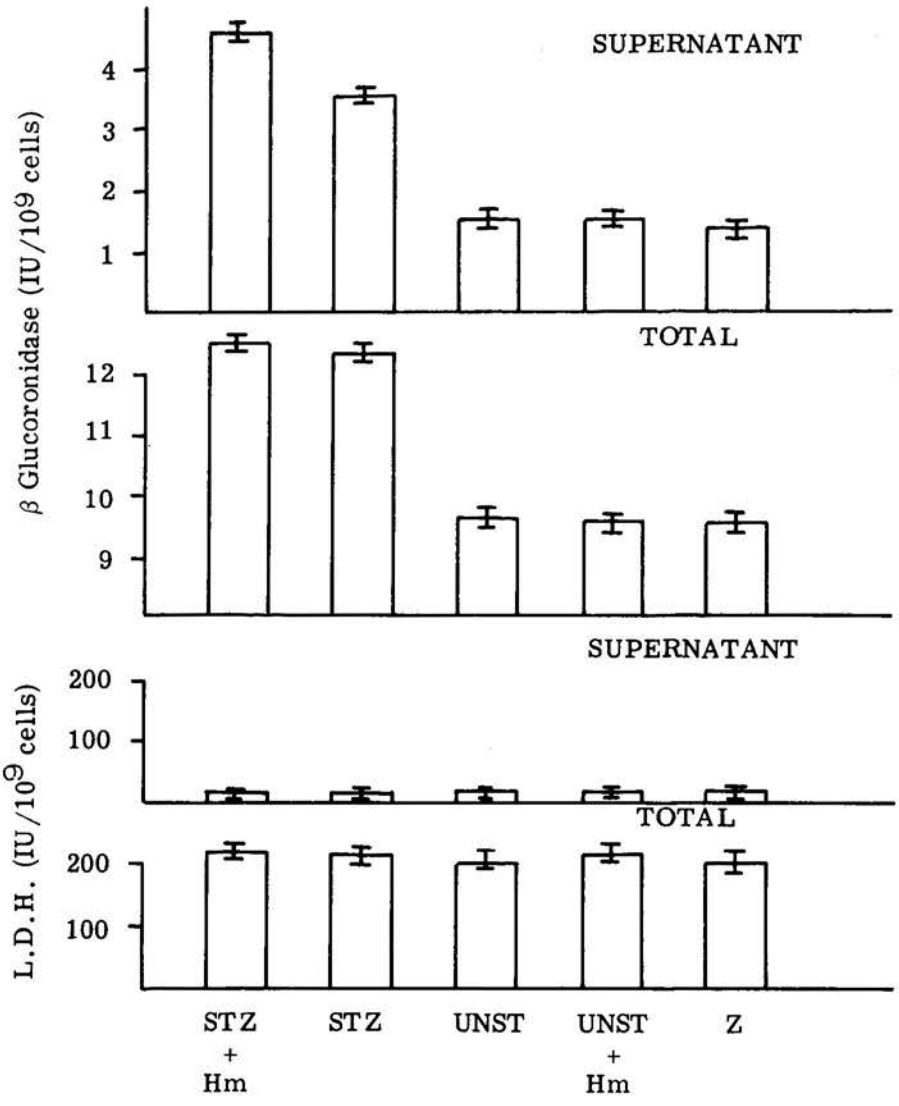


Fig. 23

β-glucuronidase and LDH release from guinea pig alveolar macrophages. Cells were treated with STZ in the presence and absence of histamine. As control, unstimulated cells were also examined in the presence and absence of histamine.

The bars represent the mean values (± 1 S.E.) of 10 experiments (β-glucuronidase) and six experiments (LDH).

2.2 Effect of cytochalasin B

The effect of cytochalasin B (5 μg/ml) on enzyme release by alveolar macrophages stimulated with STZ in the presence or absence of histamine is shown in Table X.

Cytochalasin B increased the release of β-glucuronidase in STZ-treated cells ($p < 0.02$).

The drug had a slight effect ($p < 0.05$) on the total amount of enzyme generated by STZ-stimulated cells. The enhancement of β-glucuronidase release in STZ-stimulated cells in the presence of histamine was only detected when cells were previously incubated with cytochalasin B. In the absence of cytochalasin B, histamine did not have an observable effect on STZ-treated cells.

Cytochalasin B had no apparent effect on unstimulated cells either in the presence or absence of histamine.

LDH release was not increased by the drug even when concentrations of 10 μg/ml were used.

2.3 Effect of theophylline

Theophylline at doses of 5×10^{-4} mol.l^{-1} had no effect on enzyme release by the alveolar macrophage either in the presence or absence of cytochalasin B, under the same experimental conditions described above (Table X).

2.4 Effect of H-RSAZ

The capacity of H-RSAZ to stimulate the release of lysosomal enzymes from alveolar macrophages incubated with cytochalasin B was studied and the results shown in Fig. 24. STZ was used as a positive control.

H-RSAZ did not enhance the release of β-glucuronidase and it had no effect on the residual enzyme. The amount of

Drug	Concentration	β-glucuronidase (I.U./ 10^9 cells) Supernatant Unstim	Supernatant STZ	Total Unstim	Total STZ	LDH (I.U./ 10^9 cells) Supernatant Unstim	Supernatant STZ
None	-	0.94 ± 0.25	3.01 ± 0.15	7.11 ± 0.52	9.79 ± 0.18	8.7 ± 0.35	7.6 ± 3.1
Histamine	1×10^{-4} M	1.28 ± 0.21	3.20 ± 0.20	10.01 ± 1.12	10.05 ± 0.67	7.2 ± 1.4	7.3 ± 3.3
Cytochalasin B	5 μg/ml	1.24 ± 0.53	3.64 ± 0.12	7.80 ± 0.97	12.02 ± 0.87	8.4 ± 2.5	6.4 ± 1.5
Cytochalasin B + Histamine	5 μg/ml + 1×10^{-4} M	1.43 ± 0.09	4.46 ± 0.01	8.80 ± 0.54	10.71 ± 0.22	8.6 ± 2.4	6.9 ± 1.2
Theophylline	5×10^{-4} M	1.14 ± 0.02	3.57 ± 0.27	8.23 ± 0.86	11.67 ± 0.25	6.4 ± 2.5	8.2 ± 3.5
Theophylline + Histamine	5×10^{-4} M + 1×10^{-4} M	1.19 ± 0.20	3.24 ± 0.14	9.52 ± 0.77	9.99 ± 0.43	8.3 ± 4.0	6.7 ± 3.1
Cytochalasin B + Theophylline	5 μg/ml + 5×10^{-4} M	1.28 ± 0.37	3.51 ± 0.19	8.26 ± 0.93	10.65 ± 0.11	9.6 ± 2.7	9.0 ± 1.5
Cytochalasin B + Theophylline + Histamine	5 μg/ml + 5×10^{-4} M + 1×10^{-4} M	1.00 ± 0.23	3.98 ± 0.29	7.54 ± 0.85	10.20 ± 0.40	10.7 ± 2.5	8.8 ± 1.3

TABLE X

The release of β-glucuronidase and lactic dehydrogenase by unstimulated and STZ activated guinea pig alveolar macrophages in the presence of histamine, cytochalasin B and theophylline. The results represent the mean values (± 1 S.E.) of two experiments. The incubation time was 2 hr.

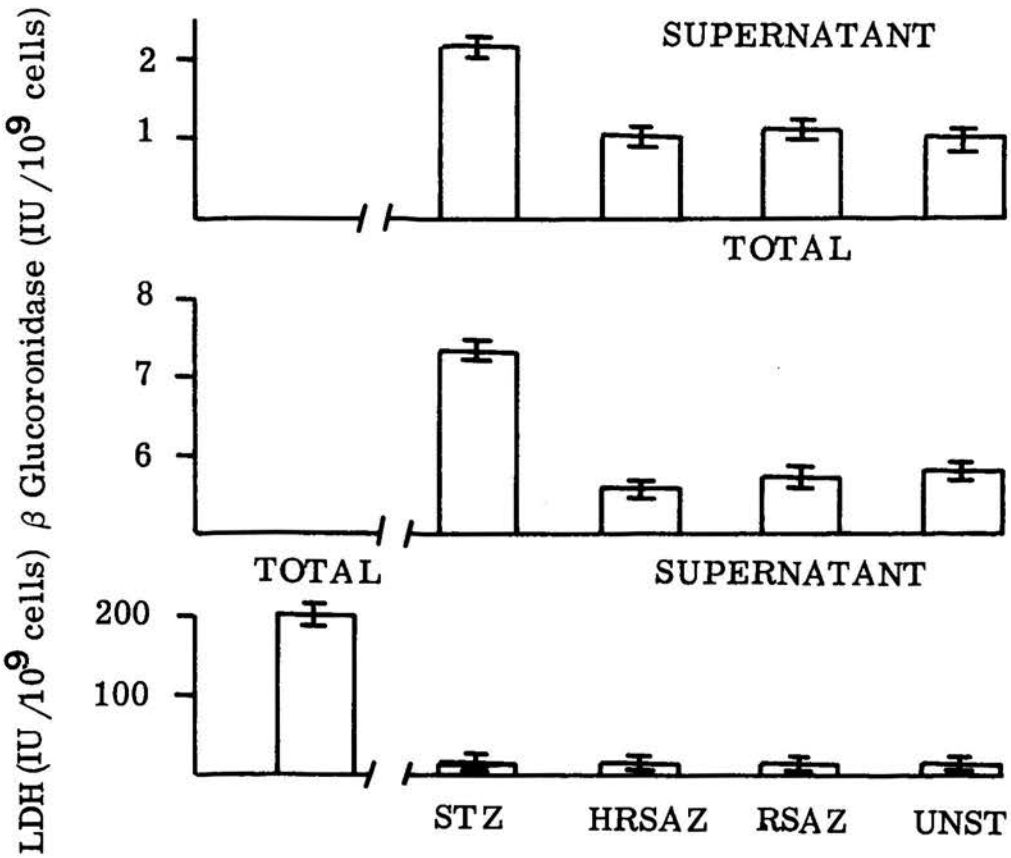


Fig. 24

The release of β-glucuronidase and lactic dehydrogenase by H-RSAZ activated guinea pig alveolar macrophages.

The bars represent the mean values (± 1 S.E.) of four experiments. The incubation time was 1 hr.

β-glucuronidase found in the supernatant was similar to controls (RSAZ and unstimulated cells) and significantly less ($p < 0.002$) than in STZ-treated cells. Also, the amount of LDH was not affected by H-RSAZ.

2.5 Effect of histamine - Dose response

Histamine at doses 10^{-4}, 10^{-5} and 10^{-6} increased the β-glucuronidase release produced by STZ-stimulated cells in a dose response manner (Fig. 25). However, the enhancement was only significant ($p < 0.05$) at 10^{-4} and 10^{-5} $mol.l^{-1}$. No effect at 10^{-6} $mol.l^{-1}$ was detected.

The total amount of β-glucuronidase was not significantly affected by the different concentrations of histamine.

Also, LDH release was not enhanced by incubation of the cells with histamine.

2.6 Effect of histamine - Time course

The release of β-glucuronidase and LDH under all experimental conditions previously described was measured at 30, 60 and 120 min incubation. The results are shown in Fig. 26.

The amount of β-glucuronidase found in the supernatant, in all samples tested, was increased with time of incubation. However, the enhancement was higher in STZ-treated cells than controls.

The release produced by STZ was always significantly higher ($p < 0.02$) than controls and the enhancement observed in the presence of histamine was paralled during incubation.

The total amount of β-glucuronidase was also increased, but not much difference was seen between 30 and 60 min. At 120 min an enhancement was observed, and the difference

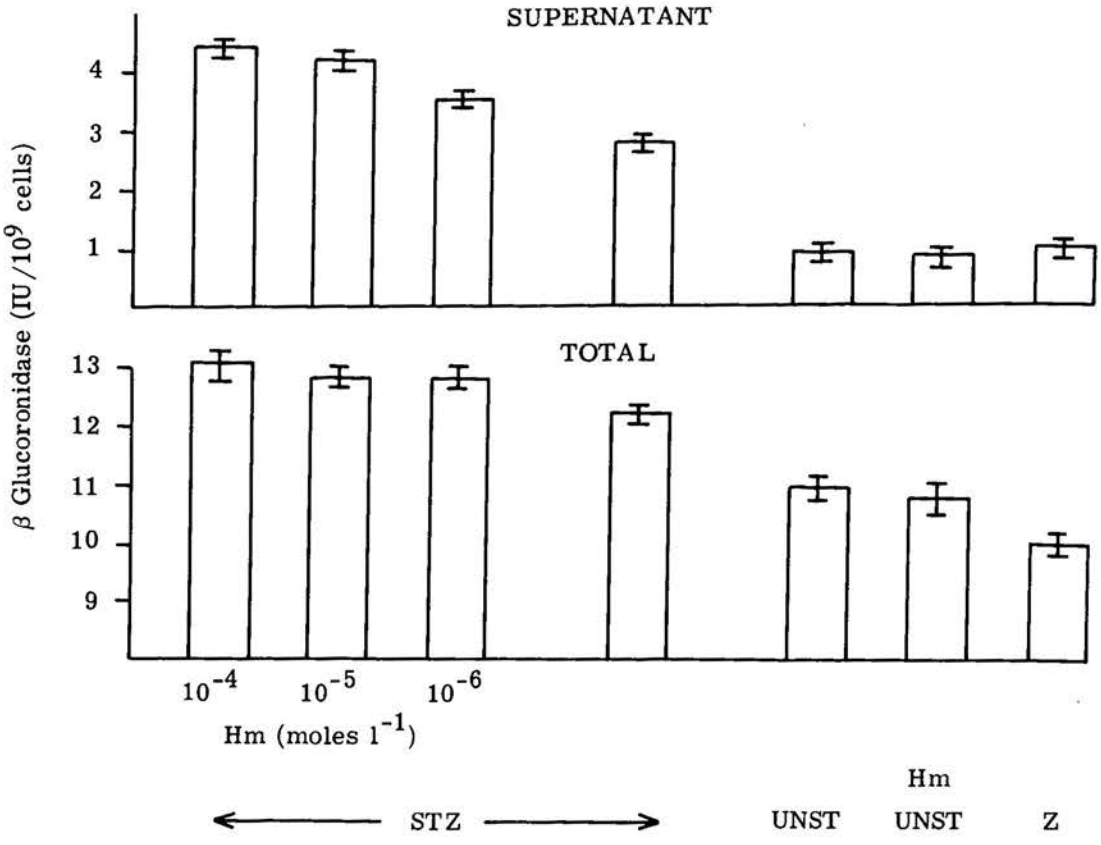


Fig. 25

The effect of "free" Hm on β-glucuronidase release by unstimulated and STZ activated guinea pig alveolar macrophages.

The bars represent the mean values (± 1 S.E.) of four experiments.

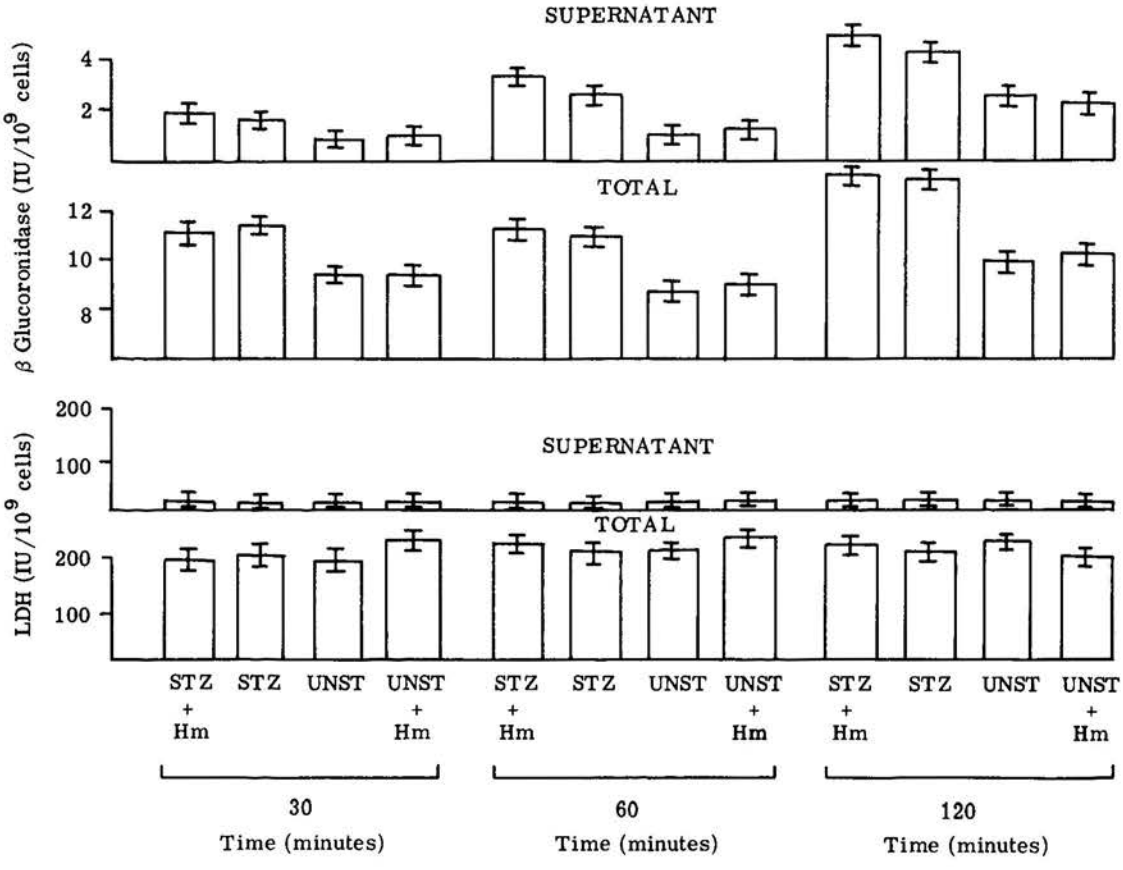


Fig. 26

The effect of incubation time on β-glucuronidase and LDH release by STZ activated guinea pig alveolar macrophages in the presence and absence of histamine.

The bars represent the mean values (± 1 SE.) of three experiments.

from controls was statistically significant ($p < 0.001$).

LDH release increased with time of incubation. However, no difference was observed due to the cell treatment.

2.7 Effect of H-RSA

This experiment was performed to determine whether H-RSA had the same effect as "free" histamine on enhancement of β-glucuronidase by STZ-treated cells. However, the control RSA produced a dose-dependent increase of this enzyme in both unstimulated or STZ-stimulated cells (Table XI). Thus, definite conclusions could not be drawn from these experiments.

2.8 Effect of histamine agonists and antagonists

In order to determine the specificity of histamine on the enhancement of β-glucuronidase release, cells were incubated in the presence of histamine agonists or pre-incubated with histamine antagonists before histamine had been added.

When cells were incubated with one H1 histamine agonist, 2-AET, and two H2 histamine agonists, 4-MeHm and Dimaprit, no significant effect on β-glucuronidase release was observed (Table XII).

Cells preincubated with the H1 histamine antagonist, chlorpheniramine, and the H2 antagonist, burimamide, released higher amounts of β-glucuronidase either unstimulated or stimulated with STZ than controls (Table XIII). The effect of the drugs was not toxic since it was not accompanied by an increase in LDH release and by a decrease in cell viability. Again, it was not possible to draw firm conclusions from these studies.

Compound	Concentration	β-glucuronidase (I.U./ 10^9 cells) Supernatant Unstim	Supernatant STZ	Total Unstim	Total STZ	LDH (I.U./ 10^9 cells) Supernatant Unstim	Supernatant STZ
None	-	0.98 ± 0.06	2.18 ± 0.11	5.86 ± 0.54	7.32 ± 0.15	15.25 ± 0.94	15.95 ± 0.95
Hm	10^{-4}	1.00 ± 0.11	2.45 ± 0.04	5.63 ± 0.48	7.30 ± 0.13	14.90 ± 0.65	14.65 ± 0.80
H-RSA	1:100	1.36 ± 0.24	2.45 ± 0.16	6.06 ± 0.52	6.35 ± 0.66	18.05 ± 1.84	17.35 ± 1.49
	1:1000	1.26 ± 0.26	2.44 ± 0.18	6.05 ± 0.27	6.29 ± 0.75	15.90 ± 0.65	14.20 ± 0.49
	1:10,000	1.34 ± 0.25	2.48 ± 0.28	5.57 ± 0.32	6.17 ± 0.65	15.95 ± 0.54	15.10 ± 0.77
RSA	1:100	1.82 ± 0.19	3.10 ± 0.13	5.73 ± 0.60	7.96 ± 1.25	12.30 ± 0.53	11.30 ± 0.96
	1:1000	1.59 ± 0.15	2.83 ± 0.07	5.32 ± 0.85	7.00 ± 0.38	13.40 ± 0.75	14.95 ± 0.17
	1:10,000	1.14 ± 0.07	2.34 ± 0.16	4.54 ± 0.44	7.28 ± 0.75	16.20 ± 0.58	13.65 ± 1.66

TABLE XI

The effect of H-RSA on β-glucuronidase and lactic dehydrogenase release by unstimulated and STZ activated guinea pig alveolar macrophages. The results represent the mean values (± 1 S.E.) of four experiments.

TABLE XII

The effect of histamine agonists, 2-AET (H1), 4-MeHm (H2) and Dimaprit (H2), on β-glucuronidase and lactic dehydrogenase release by unstimulated and STZ activated guinea pig alveolar macrophages. The results represent the mean values (± 1 S.E.) of three experiments.

Drug	Concentration (M)	β-glucuronidase (I.U./ 10^9 cells)				LDH (I.U./ 10^9 cells)	
		Supernatant		Total		Supernatant	
		Unstim	STZ	Unstim	STZ	Unstim	STZ
None	-	1.47 ± 0.17	3.23 ± 0.42	7.17 ± 0.32	9.27 ± 0.88	13.1 ± 0.7	11.2 ± 0.0
Hm	10^{-4}	1.47 ± 0.18	4.33 ± 0.16	6.84 ± 0.56	9.04 ± 0.81	15.6 ± 1.8	16.3 ± 0.2
2-AET	10^{-4}	1.71 ± 0.35	3.56 ± 0.32	7.82 ± 0.73	9.65 ± 1.19	13.7 ± 0.3	14.8 ± 0.5
	10^{-5}	-	3.28 ± 0.32	-	9.48 ± 0.60	-	17.8 ± 0.1
	10^{-6}	-	3.60 ± 0.39	-	10.38 ± 0.49	-	13.6 ± 0.2
4-MeHm	10^{-4}	1.72 ± 0.17	3.78 ± 0.43	7.35 ± 0.50	9.40 ± 1.15	13.2 ± 0.4	16.3 ± 0.2
	10^{-5}	-	3.97 ± 0.36	-	10.51 ± 1.10	-	13.6 ± 1.6
	10^{-6}	-	3.95 ± 0.50	-	9.87 ± 1.00	-	14.1 ± 0.3
Dimaprit	10^{-4}	1.54 ± 0.12	3.74 ± 0.18	7.38 ± 0.50	9.74 ± 0.59	14.9 ± 1.3	14.4 ± 0.8
	10^{-5}	-	3.98 ± 0.28	-	9.76 ± 0.95	-	13.0 ± 1.0
	10^{-6}	-	3.72 ± 0.28	-	9.11 ± 0.52	-	13.7 ± 1.5

TABLE XII

TABLE XIII

The effect of histamine antagonists on the release of β-glucuronidase and LDH by unstimulated and STZ activated guinea pig alveolar macrophages. The results represent the mean values (± 1 S.E.) of two experiments.

Drug	Concentration (M)	β-glucuronidase (I.U/ 10^9 cells)							
		Supernatant				Total			
		Unstim		STZ		Unstim		STZ	
		-	Hm 10^{-4} M	-	Hm 10^{-4} M	-	Hm 10^{-4} M	-	Hm 10^{-4} M
None	-	0.64±0.11	1.11±0.27	2.90±0.81	3.08±0.56	5.84±0.72	7.72±0.98	11.01±1.50	8.77±0.70
Chlorpheniramine	10^{-4}	1.50±0.02	-	-	4.80±1.15	9.14±2.10	-	-	11.7±2.20
	10^{-5}	-	-	-	4.20±0.44	-	-	-	11.4±1.19
	10^{-6}	-	-	-	3.39±0.53	-	-	-	9.11±0.33
Burimamide	10^{-4}	1.29±0.06	-	-	3.49±0.52	9.40±1.50	-	-	10.1±0.12
	10^{-5}	-	-	-	3.17±0.25	-	-	-	9.57±0.56
	10^{-6}	-	-	-	3.20±0.24	-	-	-	9.03±0.26
		LDH (I.U./ 10^9 cells)							
None	-	9.60±3.50	6.40±3.60	8.20±3.20	7.40±2.90	-	-	-	-
Chlorpheniramine	10^{-4}	8.10±5.70	-	-	6.80±4.80	-	-	-	-
	10^{-5}	-	-	-	7.00±4.10	-	-	-	-
	10^{-6}	-	-	-	7.80±3.60	-	-	-	-
Burimamide	10^{-4}	10.9±5.40	-	-	8.70±4.70	-	-	-	-
	10^{-5}	-	-	-	7.80±3.60	-	-	-	-
	10^{-6}	-	-	-	7.00±3.50	-	-	-	-

TABLE XIII

3.0 SUMMARY

The effect of histamine either free or conjugate bound to zymosan on enzyme release by guinea pig alveolar macrophages has been studied.

H-RSAZ, previously shown to stimulate the production of superoxide radical and chemiluminescence in a similar way to STZ, had no effect on β-glucuronidase release or in the total amount of the enzyme.

Histamine produced a dose response enhancement of β-glucuronidase release in STZ-stimulated cells. However, it was significant only at 10^{-4} and 10^{-5} $mol.l^{-1}$ and previous treatment of cells with cytochalasin B was essential. The effect was not toxic, as there was no release of LDH and no alteration of cell viability after incubation. The effect of histamine was not apparently mediated through H1- and H2-receptors since it was not possible to influence the observed effect with histamine agonists and antagonists. Moreover, the histamine antagonist drugs had a non-specific, although non-toxic, effect on unstimulated cells. Therefore, the effect of histamine on β-glucuronidase release must be interpreted with caution since it could be due to a non-specific effect and not a pharmacological action of histamine on alveolar macrophages.

CHAPTER V - DISCUSSION

1.0 RECEPTORS ON ALVEOLAR MACROPHAGES

One property of the leucocyte plasma membrane is its capacity to recognize and bind various chemical substances. These membrane markers have been termed "receptors". All white cells including alveolar macrophages probably have at least three types of "surface recognition units". These include (1) those parts of the membrane which bind opsonized particles ("immunological receptors"), (2) surface structures involved in the response to leucoattractants ("chemotactic receptors") and (3) receptors for drugs such as sympathomimetic agents and insulin ("pharmacological receptors") (Rodbell et al, 1971; Hecht et al, 1972; Cuatrecasas, 1969; Melmon et al, 1972; Berken and Benacerraf, 1966). The presence of these receptors has been established by several methods and of these, the "rosette technique", has been particularly useful in the study of receptors for immunoglobulins and complement. Such markers have now been identified as a feature of a variety of cell types including neutrophils, eosinophils, lymphocytes and various mononuclear phagocytes (Lay and Nussenzweig, 1968; Basten et al, 1972; Anwar and Kay, 1977; Berken and Benacerraf, 1966). In alveolar macrophages from different species (e.g. human, rabbit, guinea pig) the presence of receptors for the Fc portion of immunoglobulin G and the third component of complement has also been reported (Reynolds et al, 1975; Rhodes, 1975).

1.1 IgG and complement

Since, at the commencement of the work described in this thesis, the rosette technique was part of the routine

technology available in our laboratory, it seemed sensible to confirm (or otherwise) the presence of markers for IgG and complement on guinea pig alveolar macrophages. Using sheep anti-rabbit IgG, receptors for this immunoglobulin class were demonstrable on approximately 60% of normal macrophages (Fig. 3) whereas the previous studies had detected rosettes on almost all of the cells tested (Reynolds et al, 1975; Rhodes, 1975). This apparent discrepancy was probably due to differences in technical details such as time of incubation, temperature etc. In this study heterologous antibody was used and the concentration was evaluated only semi-quantitatively using an agglutination method. Therefore, the assay was probably being performed under sub-optimal conditions. The use of immunofluorescence might have improved the sensitivity. Also, this study could have been extended to include experiments with homologous antibody and identification of the immunoglobulin subclass involved. The latter could be achieved by using fluorescently labelled IgG_1 or IgG_2 or by blocking experiments with aggregates of these subtypes.

Fc receptors on the alveolar macrophage were better expressed at $0^{\circ}C$ than $37^{\circ}C$. At the higher temperature the number of rosettes decreased after 5 min incubation (Fig. 3). This may have been due either to the shedding of the plasma membrane markers or to endocytosis of the bound red cells. The latter seems the most likely explanation since after 60 min incubation red cells were visible in the cytoplasm of virtually all cells. At $0^{\circ}C$ maximal rosette formation occurred after 15 min incubation after which the percentage

of receptors remained practically unchanged. There was no evidence of endocytosis at this temperature although the maximal amount of binding was similar to that at 37°C after 5 min (Fig. 3). This suggests that the attachment of IgG to the receptor on the alveolar macrophage did not require metabolic energy.

Complement receptors were identified using fresh guinea pig serum as the complement source. When human serum was used no rosette formation was observed indicating that not all species can be used as a source of complement for detecting these receptors in the guinea pig.

The "number" of receptors on guinea pig cells was lower than previously reported for human alveolar macrophages (Reynolds et al, 1975). One explanation, in addition to species difference, is that we were using an insufficient amount of complement. However, it was not possible to increase the concentration of serum since this produced, as would be expected, an unacceptable degree of haemolysis. In order to improve the sensitivity of this assay it will be necessary to use purified complement components in which the amount of haemolytic units can be determined by effective molecular titrations.

Two unusual features of this complement-mediated rosette formation in guinea pig alveolar macrophages, compared with granulocytes and other mononuclear phagocytic cells, were that (1) although the highest percentage of rosettes was obtained at 37°C binding also occurred at 0°C (Fig. 4); and (2) there was endocytosis at 37°C. This might be explained by contamination of the IgM preparation with IgG and, therefore, Fc receptors were being formed. However, this seems unlikely since as judged by immunoelectrophoresis no IgG was detected

in the preparation. A second possibility is that endocytosis was complement-mediated, a phenomenon which has been previously reported for activated macrophages (Ehlenberger and Nussenzweig, 1977).

In conclusion, we have confirmed the previous finding that guinea pig alveolar macrophages possess receptors for IgG and complement. In view of the observed endocytosis during the formation of rosettes at 37°C it may be suggested that we were working with a "naturally activated" cell or that complement receptors are involved in the phagocytic mechanism of these cells.

1.2 Histamine rosettes

Many low molecular weight agents, including histamine, produce potent pharmacological effects on leucocytes. Therefore, such cells are believed to possess "receptors" for these agents. The initial technique used in the identification of receptors for histamine required the "insolubilization" of the amine (Melmon et al, 1972). Thus the drug was conjugated to a carrier protein and this complex was coupled to Sepharose beads. In this way the binding of human leucocytes to the beads was demonstrated since histamine could no longer enter the cell. This technique was later modified by Kedar and Bonavida (1974) who used red cells in place of Sepharose beads. They demonstrated rosette formation between various mononuclear cells and erythrocytes coated with H-RSA (Saxon et al, 1977). The general procedure involves the chemical conjugation of the histamine primary amine to carboxyl groups on the carrier protein using a coupling agent such as cyanogen bromide or, as in the present study, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (ECDI).

This coupling agent has been demonstrated to react with the carboxyl group of the carrier protein to form acid anhydride, which binds the histamine free amino group in an amide linkage (Khorana, 1953). It is possible that the highly reactive carbodiimide may also react directly with histamine through an amino group forming guanidine or with the carboxyl group of the protein to form urea (Khorana, 1953). However, any by-products of the reaction would probably be lost during the dialysis. Furthermore, it was previously reported that the histamine in the conjugate was unchanged. Thus the drug was characterized either by fractionation from the amino acids and later quantification or by tracing the reaction with labelled histamine. Both methods gave similar results demonstrating that between 30-40 moles of histamine were bound to one mole of RSA (Weinstein et al, 1973). Moreover, the integrity of the histamine moiety in the conjugate was confirmed by showing that it retained its biological activity on the isolated guinea pig ileum (Fig. 27).

In the present study the coupling of the conjugate to the cell marker was similar to that described by Kedar and Bonavida (1974), although some modifications were performed. Their technique used sheep red blood cells as cell markers. In my experience these were more susceptible to lysis than ox red cells. Also, formalized ox erythrocytes were even more resistant to haemolysis than untreated cells. Furthermore, it was found that with 20 mg/ml ECDI the degree of haemolysis was consistently less than with the dose recommended in the original technique (40 mg/ml) for use with sheep cells (Kedar and Bonavida, 1974). Also, the conjugate was diluted before coupling to the erythrocyte, since histamine in high

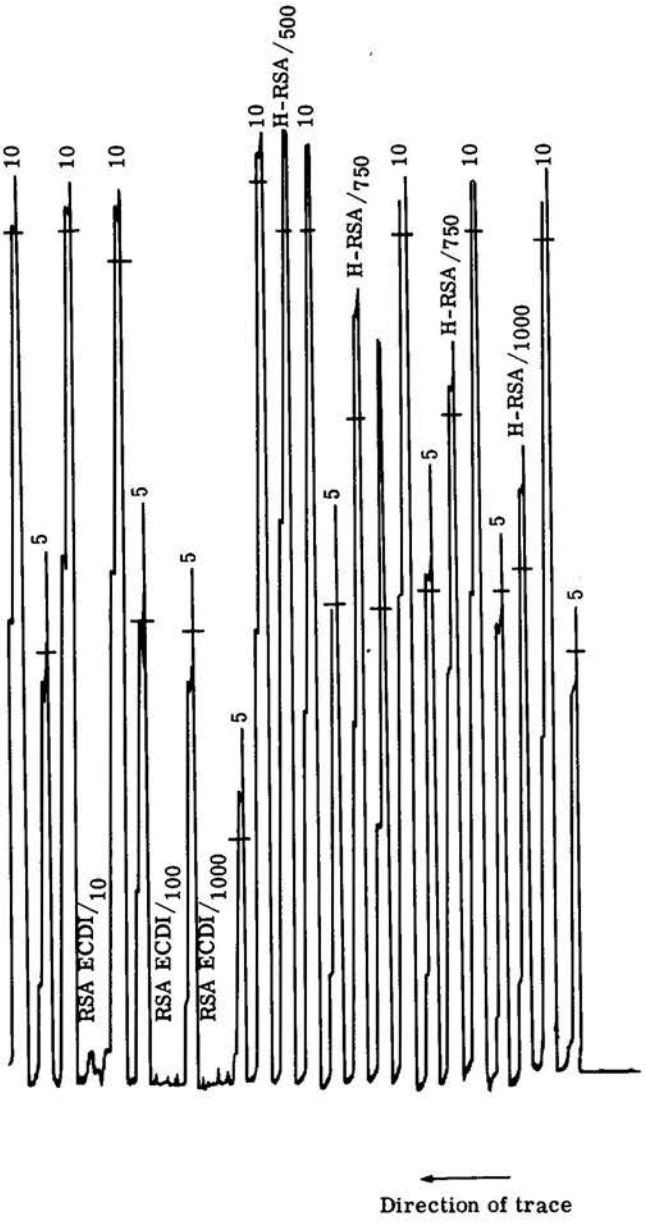


Fig. 27

Bioassay of histamine using the contraction of guinea pig ileum. Vertical axis = degree of contraction. Standard doses of 5 and 10 nanograms of histamine per ml were used throughout the experiment. H-RSA and RSA_{ECDI} were tested at the dilutions shown.

concentrations produced haemolysis. In preliminary studies, when the concentration of ECDI was 40 mg/ml, the number of rosettes formed was over 80% even with dilutions of the conjugate of 1:80 (Fig. 5). This effect was probably due to the increase of the concentration of the conjugate and, therefore, of histamine bound to the red cell. No rosette formation was observed when the control RSA was bound to the erythrocytes (Fig. 5) indicating that the high percentage of rosettes formed was due to an increase in the amount of histamine. Therefore, all guinea pig alveolar macrophages possess histamine receptors. However, the affinities of their receptors for the drug varies. One explanation for this is that due to the heterogeneity of the alveolar macrophage population some cells may possess receptors in greater numbers and with greater avidity than others and, therefore, are able to bind lower concentrations of histamine. Also, the receptors for histamine may increase in number or affinity for the amine in relation to the state of maturity or degree of activation of these cells. In this respect, the acquisition of histamine receptors by lymphoid cells after stimulation with antigen has been reported (Plaut et al, 1973b).

When the amount of ECDI was reduced to 20 mg/ml fewer rosettes were formed. A dose response with the dilution of the conjugate was observed. A gradual decrease in the number of rosettes from the highest concentration of histamine (dilution of H-RSA 1:20) down to a dilution of 1:80(Figs. 6 and 7). Further small decreases in the concentration of conjugate produced significantly fewer rosettes indicating that a minimal threshold of histamine concentration was required to bind the receptor.

Apparently there was no metabolic energy requirement involved in the binding of histamine to the receptor since the percentage of rosettes formed was the same whether the incubation was performed at 37°C or 0°C (Fig. 9). Another characteristic of this binding was that the rosettes were formed almost instantaneously and their number remained constant during incubation (Fig. 8). These two features differentiate the alveolar macrophage histamine receptors from those for immunoglobulin and complement processes, the binding to which was temperature- and time-dependent. The latter are closely involved with the phagocytic process of the alveolar macrophage. It is not possible to say whether histamine receptors are involved in the phagocytic function of these cells since detailed experiments to test phagocytic ability were not performed. Although in rosette studies no red cells were observed inside the alveolar macrophage, or other mononuclear phagocytic cells, and the percentage of rosettes did not diminish during incubation up to 30 min at 37°C (one experiment) (Fig. 9), later studies using electron microscopy showed some ingestion of H-RSAZ when cells were incubated with these particles for 1 hr at 37°C (Plate 4).

No definite conclusions can be drawn about whether the cell membranes of the various leucocytes studied bind free histamine as opposed to histamine bound as a conjugate. It is possible that the receptors only recognize histamine in its "insoluble" form. The conjugate may bind to a "rigid receptor" on the plasma membrane which, unlike receptors for complement and IgG, does not readily alter its configuration with changes in temperature. It is possible that the structure of histamine changed during conjugation and a new

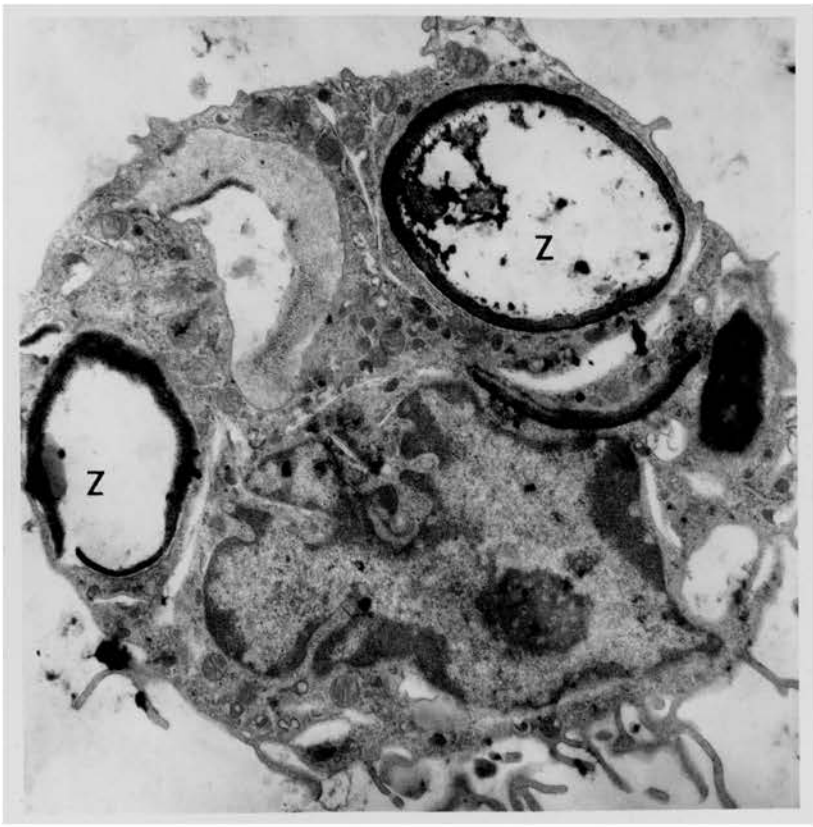


Plate 3

Electron micrograph of a guinea pig alveolar macrophage after 1 hr incubation with STZ at 37°C.

Two zymosan particles (Z) are observed to be contained within the macrophage. Magnification = x 6000.

Plate 4

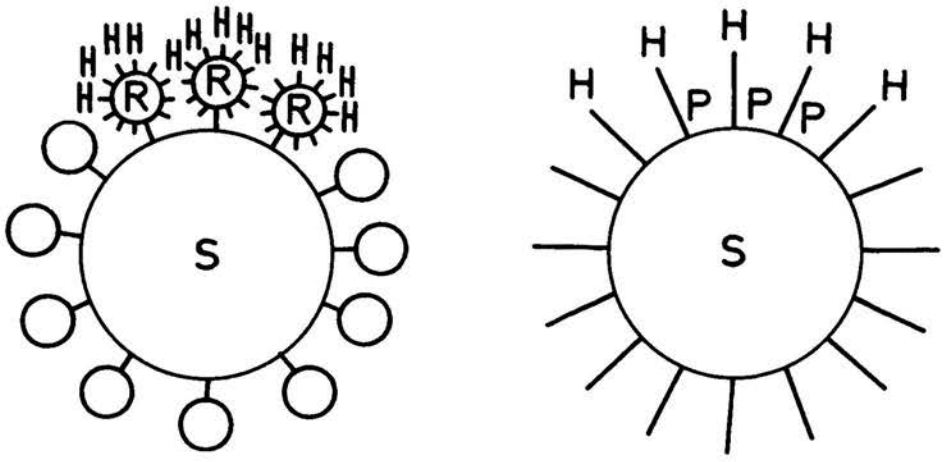
Electron micrograph of a guinea pig alveolar macrophage after 1 hr incubation with H-RSAZ at 37°C.

Zymosan particles (Z) are observed to be contained within the macrophage. Magnification = x 6000.

compound was formed. This has been postulated since no increase in cyclic AMP was observed when leucocytes were stimulated with H-RSA bound to Sepharose beads. However, H-RSA alone produced the same increase in cyclic AMP in human peripheral blood leucocytes as free histamine (Weinstein et al, 1975). This response was blocked by the histamine antagonists chlorpheniramine (H1) and burimamide (H2). However, in this study no change in the physiological properties of histamine (in the form of H-RSA and H-RSAZ) as assessed by H1-dependent contraction of guinea pig ileum was observed. Free histamine partially inhibited, and conjugated histamine totally inhibited rosette formation. Substances closely related chemically such as L-histidine, and any of the histamine catabolites, did not inhibit the reaction indicating molecular specificity in receptor binding (Fig. 10). The partial inhibition by free histamine could be explained by a change of histamine from a "monovalent" to a "polyvalent" form (Fig. 28) when conjugated to RSA and to particles (Weinstein et al, 1973). The specificity of the receptor for the amine and not for the carrier (RSA) was confirmed by the inability of the carrier to block histamine rosette formation. Furthermore, when albumin (RSA) was bound to ox red cells no rosettes were observed.

The results suggest that histamine rosettes are formed entirely at H1-receptor sites since the binding was inhibited in a dose-dependent fashion by the H1-antagonists, mepyramine and chlorpheniramine, whereas the H2-antagonists, burimamide and metiamide, had no effect. Furthermore, the reaction was blocked by the histamine H1-receptor agonist, 2-2AET (Durant et al, 1975) but not by the H2 agonist, 4-MeHm (Durant et al, 1975) and Dimaprit (Parsons et al, 1977). These findings

POLYVALENT H-RSA UNIVALENT H-COPOLYMER



H = HISTAMINE
P = RANDOM COPOLYMER OF ALANINE-TYROSINE
R = RSA
S = SEPHAROSE BEAD

Fig. 28

Schematic representation of the difference between the polyvalent H-RSA-S (left) and the univalent H-polymer-S (right).

(Taken from Weinstein et al, 1973.)

are in agreement with the original description of histamine rosette formation which was shown to be inhibited by the classical antihistamines (H1- antagonists) which included diphenhydramine, tripelennamine, antazoline and pyrilamine (Melmon et al, 1972).

"Pharmacological receptors" are usually defined by precise criteria which include a high specific binding affinity, saturability of receptors by the drug and inhibition by specific antagonists. I found that histamine possessed a high specific binding affinity, fulfilling one of these main criteria. The results also demonstrated a dose response inhibition of rosette formation by H1-histamine antagonists. However, the dose of mepyramine used in these experiments was higher than those used in other histamine activities such as inhibition of guinea pig ileum contraction. This may indicate that when histamine is bound to particles a different dose response relationship occurs to that with free histamine.

The histamine receptors of alveolar macrophages may play a role as recognition units associated with phagocytosis as it was observed that some ingestion of H-RSAZ occurred (Plate 4). They may also have a role in chemotaxis of eosinophils (Turnbull and Kay, 1976; Jones and Kay, 1977; Clark et al, 1975). Whether the binding of histamine to one type of receptor leads to events associated with immunological, chemotactic and pharmacological receptors as previously described or whether there are different receptors for mediating distinct biological events is not clear.

Under the experimental conditions described, guinea pig peritoneal macrophages and blood monocytes expressed

fewer histamine receptors than alveolar macrophages (Fig. 13). The reason for this is unclear but it has been confirmed that there are differences between and within macrophage populations (Walker, 1976; Kavai et al, 1979). It cannot easily be explained by differences in the degree of purity of cells since when peritoneal macrophages were enriched by centrifugation on Ficoll/Hypaque gradients the percentage of macrophages in both populations (alveolar and peritoneal) after Ficoll/Hypaque centrifugation was over 90%. This was confirmed by identification of the cells with non-specific esterase stain (Yam et al, 1971). Possible explanations are that the number of receptors increase with maturation or activation of the cells as discussed previously. In that condition alveolar macrophages, due to normal "contact" with the external environment, would be more active than non-stimulated peritoneal macrophages and blood monocytes. It would be of interest to test resting and activated peritoneal macrophages for their capacity to express histamine receptors. It would also be of interest to study mononuclear phagocytic cells from other tissues.

The findings that only 30-40% of lymph node cells formed rosettes is in agreement with the observation of Saxon et al (1977). These authors found T and B lymphocyte sub-populations expressed histamine receptors using the rosette technique. However, other workers (Roszkowski et al, 1977) using histamine-induced cyclic AMP elevation as a marker for functional histamine receptors, found that only T lymphocytes had receptors for the amine.

Of particular interest was the apparent inability of guinea pig eosinophils and basophils to form histamine rosettes since these cells are known to be associated with various histamine-related biological events. For instance, basophils synthesize and release this amine (Galli et al, 1976; Stewart et al, 1979), and the anaphylactic release of histamine from basophils was inhibited by histamine itself, an effect that was mediated by an H2-receptor-dependent mechanism (Lichtenstein and Gillespie, 1973, 1975). Similarly, eosinophils which respond in directional locomotion to histamine (Clark et al, 1975; Turnbull and Kay, 1976; Jones and Kay, 1977) and also presumably have a membrane recognition unit for histamine-induced chemotaxis, were unable to bind directly with stabilized histamine under the conditions described here.

The results in Table III demonstrate the presence of receptors for histamine on human alveolar macrophages. Human monocytes have also been shown to possess receptors for histamine (Smart, L., personal communication). No conclusion could be drawn from the results due to the small number of patients studied. It would be of interest to continue these studies on human cells since histamine receptors may be related to pathological conditions such as neoplastic transformation of cells. In this regard, in lymphoid cell-derived tumours in rats a lower expression or sometimes absence of histamine receptors was observed (Saxon et al, 1977).

2.0 THE RESPIRATORY BURST IN ALVEOLAR MACROPHAGES

The preliminary work in this thesis on the metabolic stimulation of guinea pig alveolar macrophages was aimed at optimizing the conditions of maximal production of the superoxide radical. At the time these studies were started there were few reports in the literature on superoxide radical production by alveolar macrophages. There was one paper in which it was claimed that there was no increase in superoxide anion during phagocytosis by guinea pig alveolar macrophages (Drath and Karnovsky, 1975) although superoxide dismutase had been found in this cell (Rister and Baehner, 1975). Also other metabolic parameters, such as stimulation of NADPH oxidase, increase in hexose monophosphate shunt and hydrogen peroxide production, had been reported to occur in alveolar macrophages during phagocytosis (Rossi et al, 1975).

There have been more recent reports on the capacity of resting alveolar macrophages to produce superoxide radical and for this to increase by at least twice during stimulation with particles (Hoidal et al, 1978a; Lowrie and Aber, 1977). This work has been confirmed in the present study.

2.1 Measurement of the superoxide radical (O_2^-)

The measurement of O_2^- used was superoxide dismutase inhibitable reduction of cytochrome C (Babior et al, 1973). This test has been shown to be reproducible and reliable and is widely accepted as a measure of this radical.

When cells were stimulated with different concentrations of zymosan, previously opsonized with serum, a dose response of O_2^- production was obtained (Fig. 14). Increasing the concentration of particles resulted in an increase in superoxide radical production indicating that an optimal cell/

particle ratio was important to maximize the observed effect.

The degree of inhibition of cytochrome C reduction in the presence of superoxide dismutase was always over 90% and often 100% was observed. These slight differences were probably due to the competitive activity of superoxide dismutase and cytochrome C for the radical. However, cytochrome C can also be reduced by other agents which might not have been affected by superoxide dismutase. Thus I have observed a reduction of the cytochrome by serum in a superoxide dismutase non-inhibitable fashion.

In general, the methodology used was the same as reported by other workers (Babior et al, 1973; Johnston et al, 1975; Weening et al, 1975). However, the concentration of the components of the reaction mixture (cytochrome C, superoxide dismutase) under the optimal conditions obtained was different to those in other reports. This was probably due to differences in the species, type and concentration of cells and the stimulating agent used. The results agreed with other reports that unstimulated alveolar macrophages produce superoxide radical but at a low level (Hoidal et al, 1978a). Using different phagocytic stimuli such as opsonized zymosan or bacteria the cells can increase their O_2^- generation by up to four- to six-fold.

The incubation medium used influenced the levels of superoxide radical produced by unstimulated cells. Thus resting cells, in a more complete medium such as M199, produced higher amounts of superoxide radical than in a simple balanced salt solution (Table VI). Similarly, serum and ovalbumin slightly stimulated the resting superoxide anion production (Fig. 15). It is not surprising that an increase

in resting O_2^- production occurred as a result of maintaining the cells in a more nutrient medium. When proteins were added pinocytosis may also have had an effect on O_2^- generation. However, when cells were fully stimulated with the appropriate concentration of opsonized zymosan particles, changing the constituents of the incubation mixture did not affect O_2^- generation. Latex stimulation was less effective in enhancing O_2^- production than STZ. However the conditions necessary to obtain maximal stimulation by latex were not fully studied.

It was previously reported that the production of superoxide radical by neutrophils stimulated with bacteria was dependent on the presence of the serum in the incubation mixture (Curnutte and Babior, 1974). The results in Fig. 15 show that serum did not affect O_2^- generation caused by zymosan particles which were already treated with serum and then washed. Therefore, the effect described by Curnutte and Babior (1974) might have been due to opsonization of the zymosan with serum and not to a soluble product of it. Later it was demonstrated that serine components including C5a and heat aggregated IgG were also able to stimulate the production of O_2^- in neutrophils (Goldstein et al, 1975a).

After prolonged preincubation at 37°C the alveolar macrophages were able to respond to STZ stimulation by increasing their O_2^- production (Fig. 16). However, the amount of O_2^- produced at resting levels and after stimulation was much less than in non-preincubated cells. This decrease in their capacity to be metabolically activated may be due to cell deterioration, death or shedding of plasma membrane.

It has been demonstrated that shedding of plasma membrane antigens in peritoneal macrophages occurred under certain conditions (Schroit et al, 1973). This phenomenon of plasma membrane shedding has also been reported in other cells (Raff, 1976). It seems unlikely that cell death was the cause of decrease in O_2^- production after prolonged pre-incubation, since tests of trypan blue dye exclusion have shown cells to be normal after 2-3 hr of cell incubation. However, this metabolic test may be more accurate in expressing any physiological changes of the cell. This was shown to be the case in bovine neutrophils (Beswick and Slater, 1978).

In conclusion, these preliminary experiments showed that STZ was able to stimulate the production of O_2^- in guinea pig alveolar macrophages. A reproducible and reliable method was developed using optimal conditions for the stimulation of these cells.

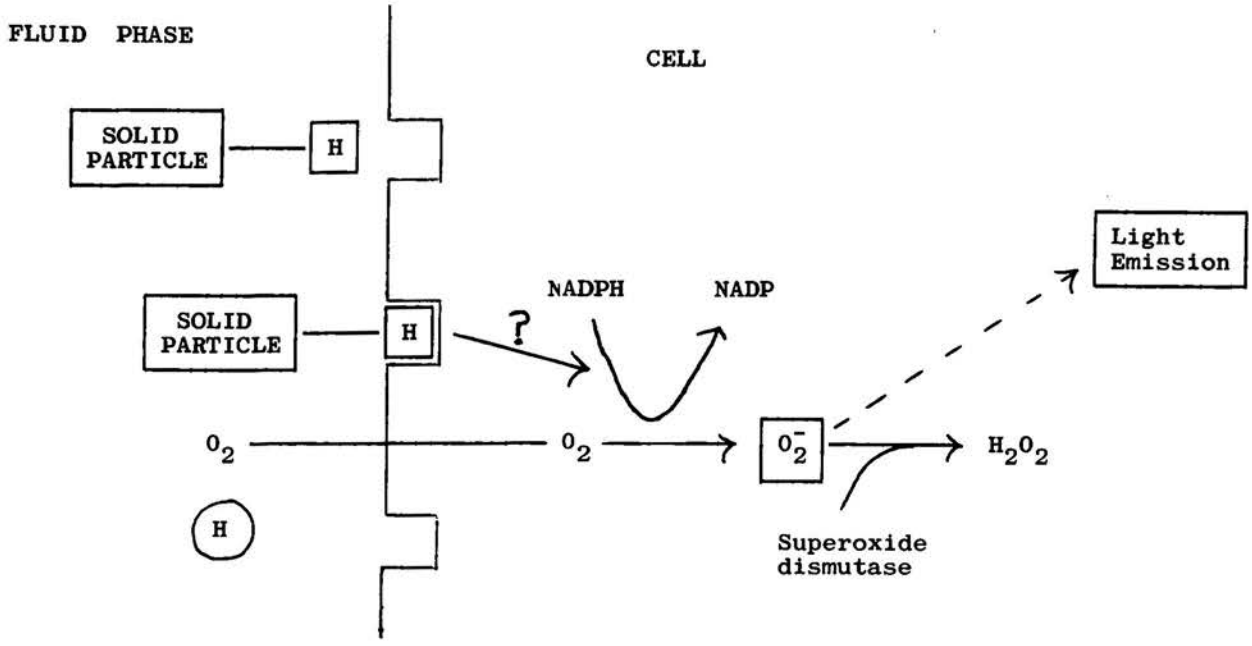
2.2 Stimulation by histamine

A series of experiments were carried out to determine whether histamine has a role to play as a modulator of oxidative activity. O_2^- generation was chosen as a suitable parameter of study of these investigations. At the beginning of these experiments the effect of preincubation of alveolar macrophages with histamine on O_2^- generation was studied. The results showed a significant dose responsive inhibition of the enhancement of O_2^- generation caused by histamine (Table VII). However, resting and phagocytosing O_2^- generation were only significantly affected at the highest concentration where the former was increased and the latter decreased. It is possible that free histamine stimulates the respiratory

burst by a small amount thereby decreasing subsequent stimulation by STZ to a similar degree.

In a pilot experiment it was shown that when cells were stimulated with H-RSA bound to ox red cells an increase in O_2^- production was obtained. However, this method was expensive in terms of the quantity of red cells used and the haemolysis produced interfered with the spectrophotometric measurements. In addition, it was probable that agents released from the red cells reduced cytochrome C thereby adversely influencing the assay system.

Although the degree of stimulation by H-RSA was higher than RSA, the response was of a much lower magnitude than even small amounts of STZ (Fig. 17). However, an additive effect was obtained when both H-RSA and STZ were used in stimulating the cells. It was only when H-RSA was bound to zymosan that the amount of superoxide radical generated was comparable to that achieved with STZ (Fig. 18). Zymosan-treated histamine may be structurally identical to the ox cells bound conjugate used in the identification of receptors. Therefore, it is possible that the conjugate has to be bound to a bigger carrier in order to bring about respiratory burst activity. In this way the histamine-coated particles might attach to receptors and trigger the metabolic events (Fig. 29), while H-RSA alone would probably either diffuse into the cell or attach only partially or weakly to the receptor. The different pharmacological activities found between H-RSA alone and H-RSA bound to Sepharose beads have already been discussed (Weinstein et al, 1975). However, H-RSAZ and H-RSA were able to contract the isolated



HISTAMINE-COATED PARTICLES GENERATE SUPEROXIDE (O_2^-) AND CHEMILUMINESCENCE (LIGHT EMISSION) IN ALVEOLAR MACROPHAGES

Fig. 29

A schematic representation of the metabolic burst as a result of conjugated histamine binding to the cell membrane. [H] = histamine conjugated to a particle; (H) = "free" histamine.

guinea pig ileum, although the concentration of H-RSAZ needed to contract the ileum was higher than that of H-RSA. Comparisons between these two different agents with regard to their concentration effect relationship cannot readily be made. When the histamine was coated on to a particle it was likely that a considerable proportion of the histamine was not available to the receptors for guinea pig ileum contraction as the rigidity of the particle maintained much of its histamine at a distance from the receptors.

It was of interest that when cells were stimulated with H-RSAZ at different concentrations of H-RSA, the dose response obtained was directly related to the concentration of H-RSA but not to the zymosan particles (Fig. 18). These results were similar to the dose response found in rosette formation (Fig. 6). This enhancement in superoxide radical production when concentration of H-RSA was increased may be due to the binding of H-RSAZ to cells with a low affinity receptor.

The characteristic pattern of the time-course of superoxide radical production when cells were stimulated with H-RSAZ and STZ, compared to controls RSAZ, H-RSA, may be another indication that when the activation of the cells is receptor-mediated a higher and more rapid response occurs (Fig. 19). This suggests that both agents (i.e. histamine and complement) are able to attach to their appropriate cell receptors when carried by suitable particles. H-RSA alone may also attach to the cell membrane since it was able to totally block histamine rosette formation. However, the attachment is probably not sufficiently strong to trigger

cellular activation. With the controls (RSA alone or RSAZ) the mechanism of stimulation of superoxide radical production is possibly different. Perhaps non-specific (non-receptor-mediated) attachment occurs and even subsequent endocytosis of these compounds.

Other carrier particles could substitute for zymosan. These included Sephadex G-10, Sepharose 4B, Amberlite G-50 and latex (Table IX). These agents, by themselves, or coated with RSA, gave a lower response than that observed in the presence of H-RSA. In general, the amount of superoxide dismutase inhibitable cytochrome C reduction was higher when zymosan was the carrier. Apparently there was an inverse relationship between particle diameter and the amount of superoxide radical produced (except latex). However, the most probable explanation for the superiority of zymosan is that the number of particles was less when larger carriers were used (since the concentration was calculated by weight). Therefore, it is probable that using the optimal cell/particle ratio a similar amount of superoxide radical could have been produced.

Chemiluminescence, which also accompanies the respiratory burst of phagocytic cells (Babior, 1978), occurs in alveolar macrophages treated with H-RSAZ (Fig. 22). This effect was both time- and dose-dependent. No light emission was observed with the controls. The reaction started almost instantaneously but the peak of chemiluminescence was observed after 8-10 min of incubation. The light emitted by phagocytic cells during the stimulation of the respiratory burst is probably due to the production of

several different processes (Babior, 1978). The results, however, showed that superoxide dismutase gave complete inhibition of chemiluminescence stimulated by H-RSAZ and STZ, indicating a close relationship between O_2^- and light emission. This finding is in agreement with some reports using human neutrophils (Hatch et al, 1978). Only 70% inhibition of light emission by superoxide dismutase was found in human monocytes and granulocytes (Johnston et al, 1976; Nelson et al, 1976). Apparently O_2^- is involved in chemiluminescence produced at the beginning of the reaction and H_2O_2 in the later stages (Cheson et al, 1976). Because of the low level light emission it was necessary to add luminol to the reaction. This compound amplifies the levels of chemiluminescence measured but does not modify the time-course and dose-response of the agents studied.

The stimulation of the respiratory burst by H-RSAZ was probably through an H1 histamine effect since the reaction was partially blocked in a dose-dependent way by the H1 histamine antagonists, chlorpheniramine and mepyramine. The inhibition of O_2^- production with the H2 histamine antagonists, burimamide and metiamide, was insignificant (Fig. 20).

Inhibition of O_2^- production by the H1 histamine antagonists was only observed when cells were stimulated with H-RSAZ and not with STZ (Fig. 21). This suggests that it had a specific effect on the histamine receptor rather than a more general effect on the plasma membrane. Furthermore, the lack of effect of histamine antagonists on O_2^- production by unstimulated cells would indicate that it

was unlikely that the drugs *per se* affected the respiratory burst.

In conclusion, evidence has been provided that the conjugate (H-RSA) bound to a carrier stimulated the respiratory burst in both human and guinea pig alveolar macrophages. This increase in the metabolic activity was demonstrated by the production of O_2^- and chemiluminescence. The effect was probably mediated through an H1 histamine receptor located in the plasma membrane.

3.0 THE EFFECT OF HISTAMINE ON ENZYME RELEASE BY ALVEOLAR MACROPHAGES

The release of lysosomal enzymes by polymorphonuclear cells and macrophages, when exposed to phagocytic and non-phagocytic stimuli, has previously been reported (Weissmann et al, 1971a,c; Zurier et al, 1973a,b; Ackerman and Beebe, 1974; Cardella et al, 1974).

In the experiments described in this thesis β-glucuronidase was chosen as the lysosomal enzyme for study since it had been previously shown to be released by guinea pig alveolar macrophages during phagocytosis (Ackerman and Beebe, 1974). Also, the assay for this enzyme is simple and reliable. Its activity was measured both in the extracellular medium (as a measure of enzyme release) and in the "pellet" in order to calculate the percentage release. The cytoplasmic enzyme, lactic dehydrogenase (LDH), was also measured in the extracellular fluid as an indicator of cell damage.

When guinea pig alveolar macrophages were incubated with serum-treated zymosan there was release of lysosomal β-glucuronidase but not of LDH, thus confirming previous reports (Ackerman and Beebe, 1974) (Fig. 23). An increase in the total amount of β-glucuronidase, but not LDH, was also observed. The production of enzymes after endocytic stimuli was previously shown in peritoneal macrophages (Cohn and Benson, 1965b). Many workers have expressed enzyme release in terms of percentage of total enzyme activity. In view of the finding that endocytic stimuli could increase the total amount of lysosomal enzyme, it

was considered preferable to express enzyme release in absolute terms. Non-opsonized zymosan had no effect on the total amount of enzyme or on its release into the incubation medium. The different effect on lysosomal enzyme production and release shown by STZ and zymosan cannot be explained by differences in the phagocytic rate since all experiments were performed in the presence of cytochalasin B. This fungal metabolite inhibits endocytosis but not attachment of the particles to the cell (Malawista, 1971; Allison et al, 1971). Therefore, STZ, but not zymosan, can bind to the macrophage through their complement receptors thereby activating the production and release of lysosomal enzymes. Thus the purified guinea pig complement component, C3b, has been shown to interact with guinea pig and mouse peritoneal macrophages in culture to induce a dose- and time-dependent release of lysosomal enzymes into the medium (Schorlemmer et al, 1976; Schorlemmer and Allison, 1976). Previous workers have reported that cytochalasin B increases lysosomal enzyme release from macrophages incubated with a phagocytic stimuli. In unstimulated cells the effect was observed only after 24 hr of incubation (Davies et al, 1973). These findings have been confirmed. A significant increase in β-glucuronidase release in cytochalasin B-treated cells stimulated with STZ, and no increase in unstimulated cells, was obtained after 2 hr incubation (Table X).

In ten experiments performed under the same experimental conditions histamine, at a high dose (10^{-4} mol.l^{-1}), increased the release of β-glucuronidase in STZ-stimulated

cells but not in unstimulated alveolar macrophages (Fig. 23). The effect of histamine was shown to be dose- and time-dependent (Figs. 25 and 26). The total amount of β-glucuronidase was not changed indicating that histamine only facilitated the release of the enzyme and did not stimulate its production (Fig. 23). This increase was probably not due to cell damage since no increase in LDH release was observed (Fig. 23). It was of interest to observe that the increase in β-glucuronidase produced by histamine was only demonstrated when cells were previously treated with cytochalasin B. In the absence of this fungal metabolite no significant difference in STZ-stimulated cells in the presence or absence of histamine was observed (Table X). The effect of histamine on guinea pig alveolar macrophages was exactly the opposite to that found in human neutrophils (Zurier et al, 1974). When neutrophils were pretreated with cytochalasin B, histamine (through its H2-receptor) decreased the release of β-glucuronidase following incubation with serum-activated zymosan particles (Busse and Sosman, 1976). Several possibilities, in addition to the difference in cell types, may explain the apparent discrepancy between the findings of Busse and Sosman (1976) and those described in this thesis. The factors which regulate lysosomal enzyme release from phagocytic cells will be considered first. The release of lysosomal enzymes from cells during phagocytosis depends on several events which include (1) attachment and endocytosis of the particle, (2) intracellular movement of lysosomes, (3) lysosomal fusion with

the phagocytic vacuole. Cyclic nucleotides, acting probably on microtubules, may influence the fusion of lysosomes with the phagosome (Weissmann et al, 1975a,b). This effect has been widely studied in polymorphonuclear phagocytic cells. Thus cyclic AMP, and substances which increase its intracellular level including histamine, decrease lysosomal enzyme release (Weissmann et al, 1971b). Cyclic GMP and its analogues increase the release (Zurier et al, 1973; Zurier et al, 1974). Cytoplasmic microtubules are in a dynamic state of assembly and disassembly (Weissmann et al, 1975b). Cyclic GMP, and agents which increase cyclic GMP levels such as phorbol myristate acetate (PMA), deuterium oxide (D_2O) and phagocytosis of particles, favour microtubule assembly and enzyme release (Goldstein et al, 1975b; Zurier et al, 1974). Cyclic AMP, agents which increase cyclic AMP levels and drugs which disrupt microtubules such as colchicine and vinblastine, inhibited phagocytosis and enzyme release (Cox and Karnovsky, 1973; Weissmann et al, 1971b). The effect of microtubules on degranulation was demonstrated in cytochalasin B-treated cells in which the phagocytosis was inhibited. In polymorphonuclear phagocytes, colchicine, vinblastine and cyclic nucleotides had the same effect on degranulation of particle-stimulated cells (e.g. STZ, bacteria) whether or not the cells were treated with cytochalasin B (Zurier et al, 1973a, 1974; Goldstein et al, 1975b; Weissmann et al, 1975b). This indicates that the effect of microtubules on degranulation was independent of phagocytosis.

In macrophages, the phenomenon of degranulation has not been fully analysed, and the literature demonstrates

that interpretation of this process is controversial. It has been shown that colchicine disrupts microtubules and inhibits phagocytosis and the release of lysosomal enzymes even from cytochalasin B-treated guinea pig alveolar macrophages and mouse peritoneal macrophages (Ackerman and Beebe, 1975; Weissmann et al, 1971b). Contradictory reports on the effects of cyclic nucleotides on phagocytosis and enzyme release have been published. Thus in mouse peritoneal macrophages an inhibition of enzyme release, without affecting particle uptake by cyclic nucleotides (3,5 cyclic AMP, dibutyryl 3,5 cyclic AMP, 3,5 cyclic GMP and 2,3 cyclic AMP), was reported (Weissmann et al, 1971b). On the other hand, in guinea pig alveolar macrophages the 3,5 adenosine and guanosine cyclic nucleotides and theophylline had no effect on phagocytosis or release of β-glucuronidase (Ackerman and Beebe, 1975). Furthermore, in rabbit alveolar macrophages, phagocytosis of latex particles increased levels of cyclic AMP and exogenous dibutyryl cyclic AMP stimulated phagocytosis, oxygen consumption and hexose monophosphate shunt (Schmidt-Gayk, et al, 1975; Seyberth et al, 1973). In contrast, in pig polymorphonuclear leucocytes, the same authors found no change in cyclic AMP during phagocytosis and a decrease in hexose monophosphate shunt activity produced by phagocytosis, when exogenous dibutyryl cyclic AMP was added (Cox and Karnovsky, 1973; Bourne et al, 1971). Moreover, a biphasic effect of cyclic AMP on phagocytosis by mouse peritoneal macrophages has been described. Thus high concentrations of the nucleotide (10^{-4} mol.l^{-1}) decreased,

and low doses (10^{-10} mol.l^{-1}) increased phagocytosis of immune complexes (Oliveira et al, 1974).

The finding that histamine increased β-glucuronidase release in STZ-stimulated alveolar macrophages (Fig. 23), an opposite effect to that found in polymorphonuclear cells (Zurier et al, 1974), may be explained by analysing the effect of histamine on any of the different degranulation steps previously described. Histamine may act on phagocytosis, a suggestion supported by the finding that there is a stimulation of the phagocytic capacity of the cells of the "reticuloendothelial system" by histamine (Gőzsy and Kato, 1957, cited by Riley, 1963). The cells in the experiment described herein were incubated with cytochalasin B and, therefore, phagocytosis should not play a role in β-glucuronidase release. Moreover, the effect of histamine was observed only when cells were incubated with cytochalasin B.

Histamine may also act on lysosome/phagosome interaction by acting on microtubules either directly or indirectly via cyclic nucleotides. Although it is possible that histamine has a direct effect on microtubules, there is no evidence, at this time, to support this view. In polymorphonuclear cells histamine, through an H2-dependent effect, increased cyclic AMP and, therefore, inhibited enzyme release (Zurier et al, 1974; Busse and Sosman, 1976). In our studies histamine may also have acted on intracellular cyclic nucleotides either increasing cyclic GMP or cyclic AMP. Cyclic AMP increased phagocytosis in alveolar macrophages but not in polymorphonuclear cells (Seyberth et al, 1973; Schmidt-Gayk et al, 1975). These opposing

effects may also be influencing enzyme release. Thus an increase in intracellular cyclic AMP leads to an increase in enzyme release in alveolar macrophages but with polymorphonuclear cells the reverse effect occurs. It should be noted that when cells were incubated with theophylline no additive effect of this drug with histamine was observed (Table X). This would suggest that cyclic AMP is not involved in enzyme release even though a small but statistically insignificant decrease in β-glucuronidase release was observed.

The most probable explanation for the enhancement of β-glucuronidase release produced by histamine is that the amine has a non-specific effect on cell permeability. Histamine has been previously used as a method for increasing cell permeability (Diengdoh and Turk, 1967). The view is supported by the fact that histamine antagonists, chlorpheniramine (H1) and burimamide (H2), did not counteract the histamine effect (Table XIII). Histamine agonists, 2-AET (H1), 4-MeHm (H2) and Dimaprit (H2), were incapable of simulating the effect (Table XII). Therefore, the effect of histamine was probably not a specific receptor-mediated activity.

The effect of histamine on the secretion of other lysosomal enzymes, besides β-glucuronidase, was not studied. It is important to state this since selective enzyme release has been observed. For instance, when cytochalasin B-treated human polymorphonuclear cells were exposed to phorbol myristate acetate extracellular release of lysozyme but not β-glucuronidase or lactic dehydrogenase was observed. However, when cytochalasin B-treated cells

were stimulated with opsonized zymosan or C5a the addition of phorbol myristate acetate resulted in enhanced β-glucuronidase release (Goldstein et al, 1975b).

It was of interest to observe that H-RSAZ did not increase the extracellular release or intracellular production of β-glucuronidase (Fig. 24), whether the experiments were performed in the presence or absence of cytochalasin B. The reason for this is not clear since H-RSAZ was shown to attach to the cell membrane and stimulate the respiratory burst (see Section B), and also in electron microscopic studies phagocytosis of H-RSAZ particles by non-cytochalasin B-treated cells has been observed (Plate 4). This may indicate that cell properties such as attachment, phagocytosis, degranulation and metabolic activity, although closely interrelated, do not necessarily all occur together at the same time. It may be that each of these activities respond to different signals rather than a "chain reaction".

The results obtained when cells were stimulated with unbound H-RSA (i.e. in the absence of zymosan) are difficult to interpret (Table XI). RSA alone per se stimulated the extracellular release of β-glucuronidase, but not its intracellular production. This effect could possibly be due to pinocytosis of the albumin. However, an inhibition of pinocytosis by cytochalasin B has been demonstrated in peritoneal macrophages (Allison et al, 1971). Although the incubation time used in this set of experiments was only 1 hr it is unlikely that this could have affected the results since in the time-course studies effects were observed as early as 30 min.

When cells were incubated with H-RSA the extracellular release of β-glucuronidase was slightly higher than with free histamine or with non-treated cells. In STZ-stimulated cells the same amount of release was obtained. It was of interest that no increase in the total amount of β-glucuronidase was obtained when cells were stimulated with STZ in the presence of H-RSA. An inhibitory effect on enzyme synthesis may have been produced but the reasons for this are not clear. A toxic effect cannot be excluded, even though the cells excluded Trypan blue. Also, an enhancement of LDH release was not observed at the concentrations of H-RSA used. At higher concentrations of H-RSA (1:10) there was release of LDH and loss of cell viability.

In conclusion, it was found that histamine-coated zymosan (H-RSAZ) particles did not stimulate the production or release of β-glucuronidase or of cytoplasmic LDH even though it initiates the respiratory burst (Section B). In contrast, serum-coated zymosan (STZ) particles stimulated both the respiratory burst and the production and release of β-glucuronidase. When cytochalasin B-treated cells were incubated with STZ in the presence of histamine, the amine, probably by a non-specific enhancement of cell permeability, increased the release of β-glucuronidase but not of LDH.

4.0 GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

The aim of the present study was to examine the effects of histamine on the alveolar macrophage. Since histamine is a well recognized mediator of inflammation in general and of type I hypersensitivity reactions in particular and is present in high concentrations in the airways of patients with certain pathological conditions such as chronic bronchitis and asthma, this amine could have a deleterious action on alveolar macrophages. On the other hand, work in recent years has heightened our appreciation of the physiological role of histamine, particularly in terms of regulation of the immune response. For example, inhibition of antibody production (Melmon *et al*, 1974), inhibition of antigen-induced lymphocyte proliferation *in vitro* (Artis *et al*, 1975), inhibition of T cell-mediated cytolysis (Henney *et al*, 1972), inhibition of MIF release (Rocklin, 1976) have been reported. Although these actions have been demonstrated *in vitro* they may also occur *in vivo*. Thus patients treated with the H2 histamine antagonist, cimetidine, had an increase in the responsiveness of lymphocytes to phytohaemagglutinin, suggesting that their lymphocytes had blocked the H2- histamine receptors (Robertson *et al*, 1979). It is known that histamine is rapidly destroyed following administration *in vivo* (Schayer, 1959). However, it is not known for certain whether small amounts of histamine are being constantly released and destroyed, a mechanism which has been suggested in the regulation of microcirculation (Schayer, 1965). Histamine may be transported extracellularly bound to a protein as a

carrier. The capacity of histamine to bind plasma protein, a phenomenon called "histaminopexy", has been demonstrated by a number of workers (Parrot et al, 1952; Guirguis, 1967; Freeman, 1969; Gecse et al, 1972). Some aspects of the phenomenon of histaminopexy have recently been studied and it was found that about 10% of histamine binds spontaneously to plasma proteins. When the coupling agent (ECDI) was added, the binding was increased up to 20% (Diaz, P. and Jones, D.G., unpublished data). It is possible that histamine exists in vivo in both forms, i.e. free and bound to a protein and it is suggested that this would be an important area for future work; that is to say, the association between plasma proteins and histamine transport.

Histamine may act preferentially through an H1- or H2-receptor depending on whether it is presented to a cell in a free or bound form and experiments to test this hypothesis could be easily designed.

Certain H2-dependent properties of histamine have exerted their effect by enhancing intracellular cyclic AMP, e.g. inhibition of enzyme release in neutrophils (Busse and Sosman, 1976), inhibition of T cell-mediated cytolysis (Plaut et al, 1973a). It is possible that H2-receptors are predominantly intracellular (and exert their activity by an increase in cyclic AMP) whereas H1-receptors are predominantly on cell membranes. Therefore, histamine in its conjugate form (H-RSA) and bound to a particle would preferentially trigger the H1- cell membrane receptor. For instance, it has been shown that with human leucocytes, H-RSA bound to Sepharose beads did not increase cyclic AMP.

However, when cells were stimulated with H-RSA alone an enhancement in the cyclic nucleotide was observed (Weinstein et al, 1975). This finding may suggest that histamine bound to Sepharose beads did not penetrate the cells. The experiments demonstrated that alveolar macrophages only respond to H1 histamine-mediated activity with regard to membrane-associated phenomena such as the presence of receptors and the activation of the respiratory burst. On the other hand, it could be that guinea pig alveolar macrophages possess only H1-receptors. All these suggestions could be put to the test by determining whether H1- or H2-receptor activity can be detected in various cell fractions.

In the present study receptors for histamine on the alveolar macrophage (guinea pig and human) were identified and shown to be H1-dependent. It would seem important, therefore, to explore the relationship between that part of the membrane which binds histamine-coated particles and that which is involved in phagocytosis and/or chemotaxis. Thus an increase in phagocytosis by cells of the "reticulo-endothelial system" in the presence of histamine has been described (Gözcy and Kato, 1957, cited by Riley, 1963).

It was also found that H-RSAZ was phagocytosed by alveolar macrophages although the rate or amount of particle uptake is not known. It is not possible, therefore, to compare H-RSAZ with STZ or with particles coated with IgG. A thorough study of the influence of histamine on phagocytosis would be important to perform, as would a comparison of H-RSAZ with the normal opsonized particles (IgG and complement)

and the effect of free histamine on the phagocytic activity of the alveolar macrophage stimulated with opsonized particles.

Also, a study of chemotactic and chemokinetic activities of histamine on alveolar macrophages and other macrophages would be important to perform.

Further studies are necessary to understand the expression of histamine receptors on macrophages. It would be important to characterize biochemically the receptor on these cells. Also, changes of the receptor expression with activation or maturation of cells and its modification on pathological conditions such as neoplasias, should be studied.

The role of histamine on alveolar macrophage function has also to be elucidated. However, it is possible only to say that histamine in its conjugated form may metabolically activate the alveolar macrophage (human and guinea pig). This could have physiological significance since O_2^- production has been found to be cytotoxic (Simchowitz and Spilberg, 1979) and, therefore, it may have a role in the defence against malignancy. Furthermore, histamine has been found to be increased in tumour tissue (Kahlson and Rosengren, 1968). However, O_2^- may also play a role in inflammatory reactions by its toxic activity. Some anti-inflammatory drugs have been demonstrated to act by inhibiting O_2^- production (Oyanagui, 1976). Therefore, in diseases such as chronic bronchitis, in which macrophages are probably in permanent contact with high concentrations of histamine (Turnbull et al, 1977), this could produce a constant stimulation of O_2^- release with a consequent damage of the tissue.

It was found that histamine did not specifically affect β-glucuronidase release. The action was probably secondary to a non-specific increase in cellular permeability. However, I am not able to say that other lysosomal enzymes are not released by stimulation with histamine either free or as H-RSAZ. It would be of interest to study the release of neutral proteinases such as collagenase and elastase under such conditions. These enzymes may play a role in lung pathologies.

The role of macrophages in the immune response has been well studied in recent years but the role of alveolar macrophages is not yet clear. It would be of interest to study the effect of histamine on the alveolar macrophage antigen processing activity and its interaction with lymphocytes. That both cells possess a large number of histamine receptors may have important implications. This could have significance in antibody production and in the interaction with T and B lymphocytes.

In conclusion, the <u>in vitro</u> work described here indicates that histamine might act as a modulator of macrophage function which in turn may have important repercussions on our understanding of certain pathological conditions. A full understanding of the interaction between histamine and alveolar macrophages in physiological and pathological processes is yet to be determined, and a great deal of further work is necessary for its final elucidation.

BIBLIOGRAPHY

ACKERMAN, N.R. & J.R. BEEBE (1974).
Release of lysosomal enzymes by alveolar mononuclear cells.
Nature 247: 475-477.

ACKERMAN, N.R. & J.R. BEEBE (1975).
Effects of pharmacologic agents on release of lysosomal enzymes from alveolar mononuclear cells.
J. Pharmac. exp. Ther. 193: 603-613.

ACTON, J.D. & Q.N. MYRVIK (1966).
Production of interferon by alveolar macrophages.
J. Bact. 91: 2300-2304.

ALISON, W.P. (1824).
Observations on the pathology of scrofulous diseases.
Trans. Med-Chir. Soc. Edin. 1: 365-438.

ALLEN, J.M., G.M.W. COOK & A.R. POOLE (1971).
Action of Concanavalin A on the attachment stage of phagocytosis by macrophages.
Exp. Cell Res. 68: 466-471.

ALLEN, R.C., R.L. STJERNHOLM & R.H. STEELE (1972).
Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity.
Biochem. Biophys. Res. Commun. 47: 679-684.

ALLISON, A.C., P. DAVIES & S. DE PETRIS (1971).
Role of contractile microfilaments in macrophage movement and endocytosis.
Nature (New Biol.) 232: 153-155.

ALTMAN, L.C., R. SNYDERMAN, J.J. OPPENHEIM & S.E. MERGENHAGEN (1973).
A human mononuclear leukocyte chemotactic factor: characterization, specificity and kinetics of production

by homologous leukocytes.

J. Immunol. 110: 801-810.

ANDERSON, R., A. GLOVER & A.R. RABSON (1977).

The in vitro effects of histamine and metiamide on neutrophil motility and their relationship to intracellular cyclic nucleotide levels.

J. Immunol. 118: 1690-1696.

ANSFIELD, M.J., H.B. KALTREIDER, J.L. CALDWELL & F.N. HERSKOWITZ (1979).

Hyporesponsiveness of canine bronchoalveolar lymphocytes to mitogens: inhibition of lymphocyte proliferation by alveolar macrophages.

J. Immunol. 122: 542-548.

ANWAR, A.R.E. & A.B. KAY (1977).

Membrane receptors for IgG and complement (C4, C3b and C3d) on human eosinophils and neutrophils and their relation to eosinophilia.

J. Immunol. 119: 976-982.

AREND, W.P. & M. MANNIK (1972).

In vitro adherence of soluble immune complexes to macrophages.

J. exp. Med. 136: 514-531.

AREND, W.P. & M. MANNIK (1973).

The macrophage receptor for IgG number and affinity of binding sites.

J. Immunol. 110: 1455-1463.

ARMSTRONG, J.A. & P. D'ARCY HART (1971).

Response of cultured macrophages to Mycobacterium tuberculosis, with observations on fusion of lysosomes with phagosomes.

J. exp. Med. 134: 713-740.

ARTIS, W.M., HE. JONES & A.A. BLAZKOVEC (1975).
Histamine inhibition of human lymphocyte transformation.
Fed. Proc. 34: 1002.

ARUNLAKSHANA, O., J.L. MONGAR & H.O. SCHILD (1954).
Potentiation of pharmacological effects of histamine by histaminase inhibition.
J. Physiol. 123: 32-54.

ASCHOFF, L. (1924).
Das reticuloendotheliole system.
Ergeb. Inn. Med. Kinderheilk. 26: 1-118.

ASH, A.S.F. & H.O. SCHILD (1966).
Receptors mediating some actions of histamine.
Br. J. Pharmac. 27: 427-439.

AXLINE, S.G. & Z.A. COHN (1970).
In vitro induction of lysosomal enzymes by phagocytes.
J. exp. Med. 131: 1239-1260.

BABIOR, B.M., R.S. KIPNES & J.T. CURNUTTE (1973).
Biological defense mechanisms. The production by leukocytes of superoxide: a potential bactericidal agent.
J. clin. Invest. 52: 741-744.

BABIOR, B.M., J.T. CURNUTTE & R.S. KIPNES (1975).
Biological defense mechanisms. Evidence for the participation of superoxide in bacterial killing by xanthine oxidase.
J. Lab. clin. Med. 85: 235-244.

BABIOR, B.M., J.T. CURNUTTE & B.J. McMURRICH (1976).
The particulate superoxide forming system from human neutrophils: properties of the system and further evidence supporting its participation in the respiratory burst.
J. clin. Invest. 58: 989-996.

BABIOR, B.M. (1978).

Oxygen dependent microbial killing by phagocytes.

New Eng. J. Med. 298: 659-668.

BALLET, J.J. & E. MERLE (1976).

The separation and reactivity in vitro of a subpopulation of human lymphocytes which bind histamine. Correlation of histamine reactivity with cellular maturation.

Cell. Immunol. 24: 250-269.

BALDRIDGE, C.W. & R.W. GERARD (1933).

The extra respiration of phagocytosis.

Am. J. Physiol. 103: 235-236.

BASTON, A., J.F.A.P. MILLER, J. SPRENT & J. PYE (1972).

A receptor for antibody on B lymphocytes. I. Method of detection and functional significance.

J. exp. Med. 135: 610-626.

BAUDRY, M., M.P. MARTERS & J.C. SCHWARTZ (1975).

H1 and H2 receptors in the histamine-induced accumulation of cyclic AMP in guinea pig brain slices.

Nature 253: 362-364.

BEAVEN, M.A. (1976).

Histamine (second of two parts).

New Eng. J. Med. 294: 320-325.

BECKER, E.L. & H.J. SHOWELL (1974).

The ability of chemotactic factors to induce lysosomal enzyme release. II. The mechanism of release.

J. Immunol. 112: 2055-2062.

BERKEN, A. & B. BENACERRAF (1966).

Properties of antibodies cytophilic for macrophages.

J. exp. Med. 123: 119-144.

BERLIN, R.D. (1973).

Temperature dependence of nucleoside membrane transport in rabbit alveolar macrophages and polymorphonuclear leukocytes.

J. Biol. Chem. 248: 4724-4730.

BERLIN, R.D., J.M. OLIVER, T.G. UKENA & H.H. YIN (1974).

Control of cell surface topography.

Nature 247: 45-46.

BERTALANFFY, F.D. (1964).

Respiratory tissue: structure, histophysiology, cytodynamics. Part I. Review and basic cytomorphology.

Int. Rev. Cyt. 16: 233-328.

BESWICK, P.H. (1977).

Phagocytosis and its associated metabolism in bovine leukocytes.

Ph.D. Thesis, Brunel University.

BESWICK, P.H. & SLATER, T.F. (1978).

Modification by metals, sulphydryl reagents and cyanide of the particle stimulated enhancement of oxygen consumption in bovine granulocytes.

Chem. Biol. Interactions 20: 373-382.

BIANCO, C., F.M. GRIFFIN & S.C. SILVERSTEIN (1975).

Studies of the macrophage complement receptor. Alteration of receptor function upon macrophage activation.

J. exp. Med. 141: 1278-1290.

BLACK, J.W., W.A.M. DUNCAN, C.J. DURANT, C.R. GANELLIN & E.M. PARSONS (1972).

Definition and antagonism of histamine H2 receptors.

Nature 236: 385-390.

BLANDEN, R.V. (1968).
Modification of macrophage function.
J. Reticuloend. Soc. 5: 179-202.

BLOCH, H. & A.A. NORDIN (1960).
Production of tuberculin sensitivity.
Nature 187: 434-435.

BLUESTEIN, H.G. & C.W. PIERCE (1973).
Cellular requirements for development of primary anti-hapten and antibody responses in vitro.
J. Immunol. 111: 137-143.

BLUSSÉ VAN OUD ALBLAS, A. & R. VAN FURTH (1979).
Origin, kinetics and characteristics of pulmonary macrophages in the normal steady state.
J. exp. Med. 149: 1504-1518.

BOUHUYS, A., R. JÖNSSON, S. LICHTNECKERT, S.E. LINDELL, C. LUNDGREN, G. LUNDIN & T.R. RINGQUIST (1960).
Effects of histamine on pulmonary ventilation in man.
Clin. Sci. 19: 79-94.

BOURNE, H.R., K.L. MELMON & L.M. LICHTENSTEIN (1971).
Histamine augments leukocyte adenosine 3'5'-monophosphate and blocks antigenic histamine release.
Science 173: 743-745.

BOURNE, H.R., R.I. LEHRER, M.J. CLINE & K.L. MELMON (1971).
Cyclic 3'5' adenosine monophosphate in the human leukocytes: synthesis, degradation and effects on neutrophil candidacidal activity.
J. clin. Invest. 50: 920-929.

BOURNE, H.R., L.M. LICHTENSTEIN, K.L. MELMON, C.S. HENNEY, Y. WEINSTEIN & G.M. SHEARER (1974).
Modulation of inflammation and immunity by cyclic AMP.
Science 184: 19-28.

BOWDEN, D.H. & I.Y.R. ADAMSON (1972).

The pulmonary interstitial cell as immediate precursor of the alveolar macrophage.

Am. J. Path. 68: 521-528.

BØYUM, A. (1968).

Isolation of mononuclear cells and granulocytes from human blood.

Scand. J. clin. Lab. Invest. 21, Suppl. 97: 77-89.

BRAIN, J.D. (1970).

Free cells in the lungs. Some aspects of their role, quantitation and regulation.

Arch. Intern. Med. 126: 477-487.

BRIGGS, R.T., M.L. KARNOVSKY & M.J. KARNOVSKY (1977).

Hydrogen peroxide production in chronic granulomatous disease: a cytochemical study of reduced pyridine nucleotide oxidases.

J. clin. Invest. 59: 1088-1098.

BRIMBLE, M.J. & D.I. WALLIS (1973).

Histamine H1 and H2 receptors at a ganglionic synapse.

Nature 246: 156-158.

BROCKLEHURST, W.E. (1960).

The release of histamine and formation of slow reacting substance (SRS-A) during anaphylactic shock.

J. Physiol. 151: 416-435.

BUSSE, W.M. & J. SOSMAN (1976).

Histamine inhibition of neutrophil lysosomal enzyme release. An H2 histamine receptor response.

Science (Wash. D.C.) 194: 737-738.

CALDERON, J., R.T. WILLIAMS & E.R. UNANUE (1974).
An inhibition of cell proliferation released by cultures of macrophages.
Proc. natn. Acad. Sci. U.S.A. 71: 4273-4277.

CALDERON, J. & E.R. UNANUE (1975).
Two biological activities regulating cell proliferation found in cultures of peritoneal exudate cells.
Nature 253: 359-361.

CALDERON, J., J.M. KIELY, J.L. LEFKO & E.R. UNANUE (1975).
The modulation of lymphocyte functions by molecules secreted by macrophages. I. Description and partial biochemical analysis.
J. exp. Med. 142: 151-164.

CALKINS, C.E. & E.S. GOLUB (1972).
Direct demonstration of lymphocyte-macrophage cooperation in the absence of physical contact between the two cell types.
Cell. Immunol. 5: 579-586.

CARDELLA, C.J., P. DAVIES & A.C. ALLISON (1974).
Immune complexes induce selective release of lysosomal hydrolases from macrophages.
Nature 247: 46-48.

CHAND, N. & P. EYRE (1975).
Classification and biological distribution of histamine receptor sub-types.
Agents Actions 5: 277-295.

CHEN, C. & J.G. HIRSCH (1972).
Restoration of antibody-forming capacity in cultures of nonadherent spleen cells by mercaptoethanol.
Science 176: 60-61.

CHESON, B.D., R.L. CHRISTENSEN, R. SPERLING, B.E. KOHLER & B.M. BABIOR (1976).
The origin of the chemiluminescence of phagocytosing granulocytes.
J. clin. Invest. 58: 789-796.

CLAMAN, H.N. & D.E. MOSIER (1972).
Cell-cell interactions in antibody production.
Prog. Allergy 16: 40-80.

CLARK, R.A.F., J.I. GALLIN & A.P. KAPLAN (1975).
The selective eosinophil chemotactic activity of histamine.
J. exp. Med. 142: 1462-1476.

COHEN, A.B. & M.J. CLINE (1971).
The human alveolar macrophage: Isolation, cultivation in vitro and studies of morphologic and functional characteristics.
J. clin. Invest. 50: 1390-1398.

COHN, Z.A. & E. WIENER (1963a).
The particulate hydrolases of macrophages. Comparative enzymology, isolation and properties.
J. exp. Med. 118: 991-1008.

COHN, Z.A. & E. WIENER (1963b).
The particulate hydrolases of macrophages. II. Biochemical and morphological response to particle ingestion.
J. exp. Med. 118: 1009-1019.

COHN, Z.A. (1964).
The fate of bacteria within phagocytic cells. III. Destruction of an Escherichia coli agglutionogen within polymorphonuclear leukocytes and macrophages.
J. exp. Med. 120: 869-883.

COHN, Z.A. & B. BENSON (1965a).
The in vitro differentiation of mononuclear phagocytes. I. The influence of inhibitors and the results of autoradiography.
J. exp. Med. 121: 279-287.

COHN, Z.A. & B. BENSON (1965b).
The in vitro differentiation of mononuclear phagocytes. II. The influence of serum on granule formation, hydrolase production and pinocytosis.
J. exp. Med. 121: 835-848.

COHN, Z.A. & B. BENSON (1965c).
The differentiation of mononuclear phagocytes. Morphology, cytochemistry and biochemistry.
J. exp. Med. 121: 153-169.

COHN, Z.A., M.E. FEDORKO & H.G. HIRSCH (1966).
The in vitro differentiation of mononuclear phagocytes. V. The formation of macrophage lysosomes.
J. exp. Med. 123: 757-766.

COHN, Z.A. (1968).
The structure and function of monocytes and macrophages.
Adv. Immunol. 9: 163-214.

COHN, Z.A. (1975).
Macrophage physiology.
Fed. Proc. 34: 1725-1729.

COHN, Z.A. (1978).
The activation of mononuclear phagocytes: Fact, fancy and future.
J. Immunol. 121: 813-816.

COX, J.P. & M.L. KARNOVSKY (1973).
The depression of phagocytosis by exogenous cyclic nucleotides, prostaglandins and theophylline.
J. Cell. Biol. 59: 480-490.

CRAPO, J.D. & D.F. TIERNEY (1974).
Superoxide dismutase and pulmonary oxygen toxicity.
Am. J. Physiol. 226: 1401-1407.

CUATRECASAS, P. (1969).
Interaction of insulin with the cell membrane: the primary action of insulin.
Proc. natn. Acad. Sci. U.S.A. 63: 450-457.

CURNUTTE, J.T. & B.M. BABIOR (1974).
Biological defense mechanisms. The effect of bacteria and serum on superoxide production by granulocytes.
J. clin. Invest. 53: 1662-1672.

DALE, H.H. & P.P. LAIDLAW (1919).
Histamine shock.
J. Physiol (Lond.) 55: 355-390.

DAUGHADAY, C.C. & S.D. DOUGLAS (1976).
Membrane receptors on rabbit and human pulmonary alveolar macrophages.
J. Reticuloend. Soc. 19: 37-45.

DAVID, J.R. & R.A. DAVID (1972).
Cellular hypersensitivity and immunity. Inhibition of macrophage migration and the lymphocyte mediators.
Prog. Allergy 16: 300-449.

DAVIES, P., A.C. ALLISON & A.D. HASWELL (1973).
Selective release of lysosomal hydrolases from phagocytic cells by cytochalasin B.
Biochem. J. 134: 33-41.

DAVIES, P., R.C. PAGE & A.C. ALLISON (1974a).
Changes in cellular enzyme levels and extracellular release of lysosomal acid hydrolases in macrophages exposed to group A streptococcal wall substance.
J. exp. Med. 139: 1262-1282.

DAVIES, P., A.C. ALLISON, J. ACKERMAN, A. BUTTERFIELD & S. WILLIAMS (1974b).
Asbestos induces selective release of lysosomal enzymes from mononuclear phagocytes.
Nature 251: 423-425.

DAVIES, P. & A.C. ALLISON (1976).
Secretion of macrophage enzymes in relation to the pathogenesis of chronic inflammation. In: Immunobiology of the Macrophage; chap. 17, 427-461.
Academic Press, New York.

DE KOCK, M.A., J.A. NADEL, S. ZWI, H.J.H. COLEBATCH & C.R. OLSEN (1966).
New method for perfusing bronchial arteries: histamine bronchoconstriction and apnea.
J. Appl. Physiol. 21: 185-194.

DESPONT, J.P. & A. CRUCHAUD (1969).
In vivo and in vitro effects of antimacrophage serum.
Nature 223: 838-839.

DIENGDOH, J.V. & J.L. TURK (1967).
Cytochemical studies on the effect of antigen on peritoneal exudate cells from guinea pigs with delayed hypersensitivity.
Int. Arch. Allergy 31: 261-273.

DRATH, D.B. & M.L. KARNOVSKY (1975).
Superoxide production by phagocytic leukocytes.
J. exp. Med. 141: 257-262.

DVORAK, H.F., S.S. SELVAGGIO, A.M. DVORAK, R.B. COLVIN, D.B. LEAN & J. RYPYSC (1974).
Purification of basophilic leukocytes from guinea pig blood and bone marrow.
J. Immunol. 113: 1694-1702.

DURANT, G.J., C.R. GANELLIN & M.E. PARSONS (1975).
Chemical differentiation of histamine H1 and H2 receptor agonists.
J. Med. Chem. 18: 905-909.

EDELSON, P.J., R. ZWEIBEL & Z. A. COHN (1975).
The pinocytic rate of activated macrophages.
J. exp. Med. 142: 1150-1164.

EHLENBERGER, A.G. & V. NUSSENZWEIG (1977).
The role of membrane receptors for C3b and C3d in phagocytosis.
J. exp. Med. 145: 357-371.

ESTENSEN, R.D., H.R. HILL, P.G. QUIE, N. HOGAN & N.D. GOLDERB (1973).
Cyclic GMP and cell movement.
Nature 245: 458-460.

EVANS, R., C.K. GRANT, H. COX, K. STEELE & P. ALEXANDER (1972).
Thymus-derived lymphocytes produce an immunologically specific macrophage-arming factor.
J. exp. Med. 136: 1318-1322.

EVANS, R. (1975).
Macrophage cytotoxicity. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 827-841.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

EYRE, P. (1973).

Histamine H2 receptors in the sheep bronchus and cat trachea: the action of burimamide.

Br. J. Pharmac. 48: 321-323.

FELDMAN, M. & J. PALMER (1971).

The requirement for macrophages in the secondary immune response to antigens of small and large size in vitro.

Immunology 21: 685-699.

FELDMAN, M. (1972).

Cell interactions in the immune responses in vitro. II. The requirement for macrophages in lymphoid cell collaboration.

J. exp. Med. 135: 1049-1058.

FISHMAN, M. (1959).

Antibody formation in tissue culture.

Nature 183: 1200-1201.

FISHMAN, M. (1961).

Antibody formation in vitro.

J. exp. Med. 114: 837-856.

FREEMAN, M.L. (1969).

Histamine binding in serum of children with asthma.

Aust. J. Paediat. 5: 133-136.

FRIDOVICH, I. (1972).

Superoxide radical and superoxide dismutase.

Acc. Chem. Res. 5: 321-326.

GALLI, S.J., A.S. GALLI, A.M. DVORAK & H.F. DVORAK (1976).

Metabolic studies of guinea pig basophilic leukocytes in short term tissue culture. I. Measurement of histamine-synthesizing capacity by using an isotopic thin layer chromatographic assay.

J. Immunol. 117: 1085-1092.

GALLILY, R. & M. FELDMAN (1967).
The role of macrophages in the induction of antibody in x-irradiated animals.
Immunology 12: 197-206.

GALLILY, R. & A.J. SCHROIT (1975).
Blocking of antimacrophage serum by shedded macrophage antigenic components. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 363-368.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

GECSE, A., J. LONOVICS, L. SZEKERES, E. ZSILINSZEKY & G.B. WEST (1972).
On the histamine-binding property of human serum.
J. Pharm. Pharmac. 24: 70-71.

GEE, J.B.L., C.L. VASALLO, M.T. VOGT, C. THOMAS & R.E. BASFORD (1971).
Peroxidative metabolism in alveolar macrophages.
Arch Int. Med. 127: 1046-1049.

GEE, J.B.L., C.L. VASALLO, P. BELL, J. KASKIN, R.E. BASFORD & J.B. FIELD (1970).
Catalase dependent peroxidative metabolism in the alveolar macrophage during phagocytosis.
J. clin. Invest. 49: 1280-1287.

GEIGER, B., R. GALLILY & I. GERY (1973).
The effect of irradiation on the release of lymphocyte activating factor (LAF).
Cell. Immunol. 7: 177-180.

GERY, I., R.K. GERSHON & B.H. WAKSMAN (1972).
Potentiation of the T-lymphocyte response to mitogens.
I. The responding cell.
J. exp. Med. 136: 128-142.

GERY, I. & R.E. HANDSCHUMACHER (1974).
Potentiation of the T-lymphocyte response to mitogens. III. Properties of the mediator(s) from adherent cells.
Cell. Immunol. 11: 162-169.

GODLESKI, J.J. & J.D. BRAIN (1972).
The origin of alveolar macrophages in mouse radiation chimeras.
J. exp. Med. 136: 630-643.

GOLDSTEIN, E., W. LIPPEIT & D. WASSHANER (1974).
Pulmonary alveolar macrophage. Defender against bacterial infection of the lung.
J. clin. Invest. 54: 519-528.

GOLDSTEIN, I., S. HOFFSTEIN, J. GALLIN & G. WEISSMANN (1973).
Mechanisms of lysosomal enzyme release from human leukocytes: Microtubule assembly and membrane fusion induced by a component of complement.
Proc. natn. Acad. Sci. U.S.A. 70: 2916-2920.

GOLDSTEIN, I.M., D. ROOS, H.B. KAPLAN & G. WEISSMANN (1975a).
Complement and immunoglobulins stimulate superoxide production by human leukocytes independently of phagocytosis.
J. clin. Invest. 56: 1155-1163.

GOLDSTEIN, I.M., S.T. HOFFSTEIN & G. WEISSMANN (1975b).
Mechanisms of lysosomal enzyme release from human polymorphonuclear leukocytes. Effects of phorbol myristate acetate.
J. Cell Biol. 66: 647-652.

GOLDSTEIN, I.M., M. CERQUEIRA, S. LIND & H.B. KAPLAN (1977).
Evidence that the superoxide-generating system of human leukocytes is associated with the cell surface.
J. clin. Invest. 59: 249-254.

GORDON, S. & Z.A. COHN (1971).

Macrophage-melanoma cell heterokaryous. IV. Unmasking the macrophage-specific membrane receptor.

J. exp. Med. 134: 947-962.

GORDON, S., J. TODD & Z.A. COHN (1974).

In vitro synthesis and secretion of lysozyme by mononuclear phagocytes.

J. exp. Med. 139: 1228-1248.

GORDON, S. (1977).

Macrophage neutral proteinases and defense of the lung.

Fed. Proc. 36: 2707-2711.

GORDON, S. (1978).

Regulation of enzyme secretion by mononuclear phagocytes: studies with macrophage plasminogen activator and lysozyme.

Fed. Proc. 37: 2754-2758.

GORDON, S., W. NEWMAN & B. BLOOM (1978).

Macrophage proteases and rheumatic diseases: regulation of plasminogen activator by thymus-derived lymphocytes.

Agents Action 8: 19-25.

GORENBERG, D.J. & R.P. DANIELE (1978).

The alveolar macrophage. Its capacity to act as an accessory cell in mitogen-stimulated proliferation of guinea pig lymphocytes.

Cell. Immunol. 36: 115-127.

GRAHAM, R.C., M.J. KARNOVSKY, A.W. SHAFER, E.A. GLASS & M.L. KARNOVSKY (1967).

Metabolic and morphological observations on the effect of surface active agents on leukocytes.

J. Cell Biol. 32: 629-647.

GREEN, G.M. & E.H. KASS (1964).
The role of the alveolar macrophage in the clearance of bacteria from the lung.
J. exp. Med. 119: 167-175.

GRENNAN, D.M., P.J. ROONEY, R.A. ST.ONGE, P.M. BROOKS, I.J. ZEITLIN & W.C. DICK (1975).
Histamine receptors in the synovial microcirculation.
Eur. J. clin. Invest. 5: 75-82.

GRIFFIN, F.M. & S.C. SILVERSTEIN (1974).
Segmental response of the macrophage plasma membrane to a phagocytic stimulus.
J. exp. Med. 139: 323-336.

GRIFFIN, F.M., Jr., C. BIANCO & S.C. SILVERSTEIN (1975a).
Characterization of the macrophage receptor for complement and demonstration of its functional independence from the receptor for the Fc portion of immunoglobulin G.
J. exp. Med. 141: 1269-1277.

GRIFFIN, F.M., J.A. GRIFFIN, J.E. LEIDER & S.C. SILVERSTEIN (1975b).
Studies on the mechanism of phagocytosis. I. Requirements for circumferential attachment of particle-bound ligands to specific receptors on the macrophage plasma membrane.
J. exp. Med. 142: 1263-1282.

GRIFFIN, F.M., J.A. GRIFFIN & S.C. SILVERSTEIN (1976).
Studies on the mechanism of phagocytosis. II. The interaction of macrophages with anti-immunoglobulin IgG-coated bone marrow-derived lymphocytes.
J. exp. Med. 144: 788-809.

GUIRGIS, H.M. (1967).
Separation of a histamine-binding fraction from normal human serum.
Int. Arch. Allergy appl. Immunol. 31: 587-593.

HAHN, H.H., D.C. CHAR, W.B. POSTEL & W.B. WOOD (1967).
Studies on the pathogenesis of fever. XV. The production of endogenous pyrogen by peritoneal macrophages.
J. exp. Med. 126: 385-394.

HALPERN, B. (1976).
Antihistaminics in immunopathologic reactions. In: Textbook of Immunopathology, vol. I; 315-328.
P.A. Miescher & H.J. Müller-Eberhard (eds.); Grune & Stratton, New York.

HARTWIG, J.H. & T.P. STOSSEL (1975).
Isolation and properties of actin, myosin and a new actin binding protein in rabbit alveolar macrophages.
J. Biol. Chem. 250: 5696-5705.

HATCH, G.E., D.E. GARDNER & D.B. MENZEL (1978).
Chemiluminescence of phagocytic cells caused by N-formyl methionyl peptides.
J. exp. Med. 147: 182-195.

HECHT, J.P., J.M. DELLACHA, J.A. SANTOME, A.C. PALADINI, E. HURWITZ & M. SELA (1972).
Lipolytic activity of bovine growth hormone bound to Sepharose beads.
Fed. Eur. Biochem. Soc. Lett. 20: 83-86.

HEISE, E.R. & Q.N. MYRVIK (1967).
Secretion of lysozyme by rabbit alveolar macrophages in vitro.
J. Reticuloend. Soc. 4: 510-523.

HENDERSON, W.R. & M. KALINER (1978).
Immunologic and nonimmunologic generation of superoxide from mast cells and basophils.
J. clin. Invest. 61: 187-196.

HENNEY, C.S., H.R. BOURNE & L.M. LICHTENSTEIN (1972).
The role of cyclic 3'5' adenosine monophosphate in the specific cytolytic activity of lymphocytes.
J. Immunol. 108: 1526-1534.

HENSON, P.M. (1971).
The immunologic release of constituents from neutrophil leukocytes. II. Mechanisms of release during phagocytosis and adherence to non-phagocytosable surfaces.
J. Immunol. 107: 1547-1557.

HIBBS, J.B., L.H. LAMBERT, Jr. & J.S. REMINGTON (1972).
Possible role of macrophage mediated monospecific cytotoxicity in tumour resistance.
Nature 235: 48-50.

HILDING, A.C. (1963).
Phagocytosis mucous flow and ciliary action.
Arch. environm. Hlth. 6: 61-73.

HILL, A.B. (1971).
Principles of Medical Statistics (9th ed.)
Oxford University Press, New York.

HIRSCH, M.S., G.W. GARY & F.A. MURPHY (1969).
In vitro and in vivo properties of antimacrophage sera.
J. Immunol. 102: 656-661.

HOIDAL, J.R., J.E. REPINE, G.D. BEALL, F.L. RASP & J.G. WHITE (1978a).
The effect of phorbol myristate acetate on the metabolism and ultrastructure of human alveolar macrophages.
Am. J. Path. 91: 469-476.

HOIDAL, J.R., G.D. BEALL, F.L. RASP, Jr., B. HOLMES, J.G. WHITE & J.E. REPINE (1978b).
Comparison of the metabolism of alveolar macrophages from humans, rats and rabbits: response to heat-killed bacteria or phorbol myristate acetate.
J. Lab. clin. Med. 92: 787-794.

HOFFMANN, M. & R.W. DUTTON (1971).
Immune response restoration with macrophage culture supernatants.
Science 172: 1047-1048.

HOLLAND, P., N.H. HOLLAND & Z.A. COHN (1972).
The selective inhibition of macrophage phagocytic receptors by antimembrane antibodies.
J. exp. Med. 135: 458-475.

HOLT, P.G. (1979a).
Alveolar macrophages. II. Inhibition of lymphocyte proliferation by purified macrophages from rat lung.
Immunology 37: 429-436.

HOLT, P.G. (1979b).
Alveolar macrophages. III. Studies on the mechanism of inhibition of T-cell proliferation.
Immunology 37: 437-445.

IGNARRO, L.J. (1974).
Regulation of lysosomal enzyme secretion. Role in inflammation.
Agents Action 4: 241-258.

IGNARRO, L.J. & W.J. GEORGE (1974).
Mediation of immunologic discharge of lysosomal enzymes from human neutrophils by guanosine 3'5' monophosphate.
J. exp. Med. 140: 225-238.

IGNARRO, L.J., T.F. LINT & W.J. GEORGE (1974a).
Hormonal control of neutrophil lysosomal enzyme release from human neutrophils.
J. exp. Med. 139: 1395-1414.

IGNARRO, L.J., R.J. PADDOCK & W.J. GEORGE (1974b).
Hormonal control of neutrophil lysosomal enzyme release. Effect of epinephrine in adenosine 3'5' monophosphate.
Science 183: 855-857.

IYER, G.Y.N., M.F. ISLAM & J.H. QUASTEL (1961).
Biochemical aspects of phagocytosis.
Nature 192: 535-541.

JOHNSTON, R.B., Jr., B.B. KEELE, Jr., H.P. MISRA, J.E. LEHMEYER, L.S. WEBB, R.L. BAEHNER & K.V. RAJAGOPALAN (1975).
The role of superoxide anion generation in phagocytic bactericidal activity. Studies with normal and chronic granulomatous disease leukocytes.
J. clin. Invest. 35: 1357-1372.

JOHNSTON, R.J., Jr. & J.E. LEHMEYER (1976).
Elaboration of toxic oxygen by-products by neutrophils in a model of immune complex disease.
J. clin. Invest. 57: 836-841.

JOHNSTON, R.J., Jr., J.E. LEHMEYER & L.A. GUTHRIE (1976).
Generation of superoxide anion and chemiluminescence by human monocytes during phagocytosis and on contact with surface-bound immunoglobulin G.
J. exp. Med. 143: 1551-1556.

JOHNSTON, R.B., Jr., C.A. GODZIK & Z.A. COHN (1978).
Increased superoxide anion production by immunologically activated and chemically elicited macrophages.
J. exp. Med. 148: 115-127.

JONES, D.G. & KAY, A.B. (1976).
The effect of anti-eosinophil serum on skin histamine replenishment following passive cutaneous anaphylaxis in the guinea pig.
Immunology 31: 333-336.

JONES, D.G. & KAY, A.B. (1977).
Chemotactic activity of guinea pig eosinophils for the ECF-A acidic tetrapeptides, histamine, histamine metabolites and the effect of H1 and H2 receptor antagonists.
Int. Arch. Allergy appl. Immunol. 55: 277-282.

JONES, T.C. (1975a).
Attachment and ingestion phase of phagocytosis. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 269-282.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

JONES, T.C. (1975b).
Phagosome-lysosome interaction with Toxoplasma. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 595-605.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

KAHLSON, G. & E. ROSENGREN (1968).
New approaches to the physiology of histamine.
Physiol. Rev. 48: 155-196.

KALTREIDER, H.B. (1976).
Expression of immune mechanisms in the lung.
Am. Rev. Resp. Dis. 113: 347-379.

KARNOVSKY, M.L., J. LASDINS & S.R. SIMMONS (1975).
Metabolism of activated mononuclear phagocytes at rest and during phagocytosis. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 423-438.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

KARRER, H.E. (1958).
The ultrastructure of mouse lung. The alveolar macrophage.
J. Biophys. Biochem. Cytol. 4: 693-699.

KATZ, D.H. & B. BENACERRAF (1972).
The regulatory influence of activated T cells on B cell responses to antigens.
Adv. Immunol. 15: 1-94

KATZ, D.H. & E.R. UNANUE (1973).
Critical role of determinant presentation in the induction of specific responses in immunocompetent lymphocytes.
J. exp. Med. 137: 967-990.

KAVAI, M., J. LACZKO & B.C. SABA (1979).
Functional heterogeneity of macrophages.
Immunology 36: 729-732.

KAY, A.B. & K.F. AUSTEN (1971).
The IgE-mediated release of an eosinophil leukocyte chemotactic factor from human lung.
J. Immunol. 107: 899-902.

KEDAR, E. & B. BONAVIDA (1974).
Histamine receptor bearing leukocytes. I. Detection of histamine receptor bearing cells by rosette formation with histamine coated erythrocytes.
J. Immunol. 113: 1544-1552.

KELLER, R. (1975).

Major changes in lymphocyte proliferation evoked by activated macrophages.

Cell. Immunol. 17: 542-551.

KHORANA, H.G. (1953).

The chemistry of carbodiimides.

Chem. Rev. 53: 145-166.

KILBURN, K.H. (1974).

Functional morphology of the distal lung.

Int. Rev. Cytol. 37: 153-270

KLEBANOKK, S.J. (1967a).

A peroxidase mediated antimicrobial system in leukocytes.

J. clin. Invest. 46: 1078.

KLEBANOFF, S.J. (1967b).

Iodination of bacteria: a bactericidal mechanism.

J. exp. Med. 126: 1063-1076.

KLEBANOFF, S.J. & C.B. HAMON (1975).

Antimicrobial systems of mononuclear phagocytes. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 507-529.

R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

KORNBERG, A. & W.E. PRICER, Jr. (1951).

Enzymatic phosphorylation of adenosine and 2,6, diamino-purine roboside.

J. Biol. Chem. 193: 481-495.

KOWNATZKI, E., G. TILL, M. GAGELMANN, G. TERWORT & D. GEMSA (1977).

Histamine induces release of an eosinophil immobilising factor from mononuclear cells.

Nature 270: 67-69.

KRAHENBUHL, J.L. & J.S. REMINGTON (1971).
In vitro induction of non-specific resistance in macrophages by specifically sensitized lymphocytes.
Infect. Immunity 4: 337-343.

KRINSKY, N.I. (1974).
Singlet excited oxygen as a mediator of the antibacterial action of leukocytes.
Science 186: 363-365.

LAUWERIYNS, J.M. & J.H. BAERT (1977).
Alveolar clearance and the role of pulmonary lymphatics.
Am. Rev. Resp. Dis. 115: 625-683.

LAY, W.H. & V. NUSSENZWEIG (1968).
Receptors for complement on leukocytes.
J. exp. Med. 128: 991-1007.

LEIBOVICH, S.J. & R. ROSS (1975).
Macrophages and anti-macrophage serum. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 151-160.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

LICHTENSTEIN, L.M. & E. GILLESPIE (1973).
Antihistamines, histamine, histamine receptors and histamine release.
Fed. Proc. 32: 1010.

LICHTENSTEIN, L.M. & E. GILLESPIE (1975).
The effects of the H1 and H2 antihistamines on "allergic" histamine release and its inhibition by histamine.
J. Pharm. exp. Ther. 192: 441-450.

LIPSKY, P.E. & A.S. ROSENTHAL (1973).
Macrophage-lymphocyte interaction. I. Characteristics of the antigen-independent binding of guinea pig thymocytes and lymphocytes to syngeneic macrophages.
J. exp. Med. 138: 900-924.

LITTLETON, C., D. KESSLER & P.M. BURKHOLDER (1970).
Cellular basis for synthesis of the fourth component of guinea pig complement as determined by a haemolytic plaque technique.
Immunology 18: 693-704.

LoBUGLIO, A. & J. REINEHARD (1970).
In vitro and in vivo modification of human macrophage receptor for IgG glogulin.
Clin. Res. 18: 409.

LOHMANN-MATTHES, M.L. (1976).
Induction of macrophage mediated cytotoxicity. In: Immunobiology of the Macrophage; 464-486.
D.S. Nelson (ed.); Academic Press, New York.

LORHMANN, H.P., L. NOVIKOVS & R.G. GRAW, Jr. (1974).
Cellular interactions in the proliferative response of human T and B lymphocytes to phytomitogens and allogeneic lymphocytes.
J. exp. Med. 139: 1553-1567.

LOWRIE, D.B. & V.R. ABER (1977).
Superoxide production by rabbit pulmonary alveolar macrophages.
Life Sci. 21: 1575-1583.

LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR & R.J. RANDALL (1951).
Protein measurement with the folin phenol reagent.
J. Biol. Chem. 193: 265-275.

McARTHUR, W.P., P. BAEHNI & N.S. TAICHMAN (1976).
Interaction of inflammatory cells and oral micro-organisms. III. Modulation of rabbit polymorphonuclear leukocyte hydrolase release response to Actinomyces viscosus and Streptococcus mutants by immunoglobulins and complement.
Infect. Immunity 14: 1315-1321.

McCLELLAND, D.B.L., R.F.M. LAI, A. FAT & R. VAN FURTH (1975).
Synthesis of lysozyme in vitro by mouse and human mononuclear phagocytes. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 475-486.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

MACKANESS, G.B. (1962).
Cellular resistance to infection.
J. exp. Med. 116: 381-406.

MACKANESS, G.B. (1964).
The immunological basis of acquired cellular resistance.
J. exp. Med. 120: 105-120.

MACKANESS, G.B. (1969).
The influence of immunologically committed lymphoid cells on macrophage activation in vivo.
J. exp. Med. 129: 973-992.

MACKANESS, G.B. (1971).
The induction and expression of cell mediated hypersensitivity in the lung.
Am. Rev. Resp. Dis. 104: 813-828.

MALAWISTA, S.E., J.B.L. GEE & K.G. BENSCH (1971).
Cytochalasin B reversible inhibits phagocytosis: functional, metabolic and ultrastructural effects in human blood leukocytes and rabbit alveolar macrophages.
Yale J. Biol. Med. 44: 286-300.

MANGANIELLO, V., W.H. EVANS, T.P. STOSSEL, R.J. MASON & M. VAUGHAN (1971).
The effect of polystyrene beads on cyclic 3'5' adenosine monophosphate concentration in leukocytes.
J. clin. Invest. 50: 2741-2744.

MANTOVANI, B., M. RABINOVITCH & V. NUSSENZWEIG (1972).
Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG).
J. exp. Med. 135: 780-792.

MARTINEZ, R.D. & J. MONTFORD (1973).
A study of the specificity of alveolar macrophage antigen(s).
Immunology 25: 197-203.

MASON, R.J., T.P. STOSSEL & M. VAUGHAN (1972).
Lipids of alveolar macrophages, polymorphonuclear leukocytes and their phagocytic vesicles.
J. clin. Invest. 51: 2399-2407.

MELMON, K.L., H.R. BOURNE, J. WEINSTEIN & M. SELA (1972).
Receptors for histamine can be detected on the surface of selected leukocytes.
Science (Wash. D.C.) 177: 707-709.

MELMON, K.L., H.R. BOURNE, J. WEINSTEIN, G.M. SHEARER, J. KRAM & S. BAUMINGER (1974).
Haemolytic plaque formation by leukocytes in vitro.

Control by vasoactive hormones.

J. clin. Invest. 53: 13-21.

MELTZER, M.S., R.N. TUCKER, K.K. SANFORD & E.J. LEONARD (1975).

Interaction of BCG activated macrophages with neoplastic and nonneoplastic cell lines in vitro: quantitation of the cytotoxic reaction by release of tritiated thymidine from prelabeled target cells.

J. natn. Cancer Inst. 54: 1177-1184.

METSCHNIKOFF, E.I. (1884).

Ueber eine Sprosspilzkrankheit der Daphnien. Beitrag zur Lehre über den Kampp der Phagocyten gegen Krankheit-serrescr.

Arch. Pathol. Anat. Physiol. Klin. Med. XCVI: 177-195.

MITCHISON, N.A. (1969).

The immunogenic capacity of antigen taken up by peritoneal exudate cells.

Immunology 16: 1-14.

MORROW, P.E. (1973).

Alveolar clearance of aerosols.

Arch. Int. Med. 131: 101-108.

MOORE, V.L. & Q.N. MYRVICK (1974).

Inhibition of normal rabbit alveolar macrophages by factor(s) resembling migration inhibition factor.

J. Reticuloend. Soc. 16: 21-26.

MOSIER, D.E. (1967).

A requirement for two cell types for antibody formation in vitro.

Science 158: 1573-1575.

MOSIER, D.E. (1975).

Low doses of DPN-D-GL, a potent hapten specific tolerogen are immunogenic *in vitro*.

Nature *257*: 141-142.

MUNDER, P.G. & M. MODOLLEL (1973).

Adjuvant induced formation of lysophosphatides and their role in the immune response.

Int. Arch. Allergy appl. Immunol. *45*: 133-135.

MYRVIK, Q.N., E.S. LEAKE & B. FARRIS (1961).

Studies on pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a high state of purity.

J. Immunol. *86*: 128-132.

NATHAN, C.F., M.L. KARNOVSKY & J.R. DAVID (1971).

Alterations in macrophage functions by mediators from lymphocytes.

J. exp. Med. *133*: 1356-1376.

NATHAN, C.F. & R.K. ROOT (1977).

Hydrogen peroxide release from mouse peritoneal macrophages. Dependence on sequential activation and triggering.

J. exp. Med. *146*: 1648-1662.

NELSON, D.S. (1973).

Production by stimulated macrophages of factors depressing lymphocyte transformation.

Nature *246*: 306-307.

NELSON, D.S. (1976).

Immunobiology of the Macrophage.

Academic Press, New York.

NELSON, R.A., J. JENSEN, I. GIGLI & N. TAMURA (1966).
Methods for separation, purification and measurement of nine components of haemolytic complement in guinea pig serum.
Immunochemistry 3: 111-135.

NELSON, R.D., E.L. MILLS, R.L. SIMMONS & P.G. QUIE (1976).
Chemiluminescence response of phagocytozing human monocytes.
Infect. Immunity 14: 129-134.

NORTH, R.J. (1978).
The concept of the activated macrophage.
J. Immunol. 121: 806-809.

OLIVEIRA LIMA, A., M.Q. JAVIERRE, W. DIAS DA SILVA & D. SETTE CAMARA (1974).
Immunological phagocytosis: effect of drugs on phosphodiesterase activity.
Experientia 30: 945-946.

OLIVER, J.M. & R.D. BERLIN (1976).
Macrophage membranes. In Immunobiology of the Macrophage; 259-273.
D.S. Nelson (ed.); Academic Press, New York.

OREN, R., A.E. FARNHAM, K. SAITO, E. MILOFSKY & M.L. KARNOVSKY (1973).
Metabolic patterns in three types of phagocytizing cells.
J. Cell Biol. 17: 487-501.

OUCHI, E., R.J. SELVARAJ & A.J. SBARRA (1965).
The biochemical activities of rabbit alveolar macrophages during phagocytosis.
Exp. Cell Res. 40: 456-468.

OYANAGUI, Y. (1976).
Inhibition of superoxide anion production in macrophages by anti-inflammatory drugs.
Biochem. Pharm. 25: 1473-1480.

PAGE, R.C., P. DAVIES & A.C. ALLISON (1974).
Pathogenesis of the chronic inflammatory lesion induced by group A streptococcal cell walls.
Lab. Invest. 30: 568-581.

PANIJEL, J. & P. CAYEUX (1968).
Immunosuppressive effects of macrophage antiserum.
Immunology 14: 769-780.

PANTALON, R.M. & R.C. PAGE (1975).
Lymphokine-induced production and release of lysosomal enzymes by macrophages.
Proc. natn. Acad. Sci. U.S.A. 72: 2091-2094.

PARROT, J.L., D.A. URQUIA & C. LABORDE (1952).
Action histaminopexique de serum humain et son pouvoir protecteur a l'epaid de l'histamine. Modification par la dialyse ou par l'addition d'histamine.
Compt. Rend. Soc. Biol. 146: 1052-1055.

PARSONS, M.E., D.A.A. OWEN, C.R. GANELLIN & G.J. DURANT (1977).
Dimaprit, S [3-(N,N-dimethylamino) propyl] isothiourea, a highly specific histamine H2 receptor agonist. Part I. Pharmacology.
Agents Action 7: 31-37.

PAUL, B.B., R.R. STRAUSS, R.J. SELVARAJ & A.J. SBARRA (1973).
Peroxidase mediated antimicrobial activities of alveolar macrophage granules.
Science 181: 849-850.

PHILLIPS-QUAGLIATA, J.M., B.B. LEVINE, F. QUAGLIATA & J.W. UHR (1971).
Mechanisms underlying binding of immune complexes to macrophages.
J. exp. Med. 133: 589-601.

PIERCE, C.W. (1969).
Immune response in vitro. I. Cellular requirements for the immune response by nonprimed and primed spleen cells in vitro.
J. exp. Med. 130: 345-364.

PIERCE, C.W. (1973).
Immune responses in vitro. VI. Cell interactions in the development of primary IgM, IgG and IgA plaque-forming cell responses in vitro.
Cell. Immunol. 9: 453-464.

PIERCE, C.W., J.A. KAPP, D.D. WOOD & B. BENACERRAF (1974).
Immune responses in vitro. X. Functions of macrophages.
J. Immunol. 112: 1181-1189.

PIESSENS, W.F., W.H. CHURCHILL & J.R. DAVID (1975).
Macrophages activated in vitro with lymphocyte mediators kill neoplastic but not normal cells.
J. Immunol. 114: 293-299.

PINKETT, M.O., C.R. COWDREY & P.C. NOWELL (1966).
Mixed hematopoietic and pulmonary origin of "alveolar macrophages" as demonstrated by chromosome markers.
Am. J. Path. 48: 859-867.

PLAUT, M., L.M. LICHTENSTEIN, E. GILLESPIE & C.S. HENNEY (1973a).
Studies on the mechanism of lymphocyte-mediated cytolysis.

IV. Specificity of the histamine receptor on effector T cells.

J. Immunol. 111: 389-394.

PLAUT, M., L.M. LICHTENSTEIN & C.S. HENNEY (1973b).

Increase in histamine receptors on thymus-derived effector lymphocytes during the primary immune response to alloantigens.

Nature 244: 284-286.

POULTER, L.W. & J.L. TURK (1975a).

Studies on the effect of soluble lymphocyte products (lymphokines) on macrophage physiology. I. Early changes in enzyme activity and permeability.

Cell. Immunol. 20: 12-24.

POULTER, L.W. & J.L. TURK (1975b).

Studies on the effect of soluble lymphocyte products (lymphokines) on macrophage physiology. II. Cytochemical changes associated with activation.

Cell. Immunol. 20: 25-32.

PRUZANSKY, J.J., I.M. SUSZKO & R. PATTERSON (1976).

Uptake and catabolism of antigen by alveolar macrophages of dogs with respiratory hypersensitivity. Processing of antigen by alveolar macrophages.

Clin. exp. Immunol. 25: 165-169.

RABINOVITCH, M. (1967).

The dissociation of the attachment and ingestion phases of phagocytosis by macrophages.

Exp. Cell Res. 46: 19-28.

RABINOVITCH, M. & M.J. DE STEFANO (1973a).

Particle recognition by cultivated macrophages.

J. Immunol. 110: 695-701.

RABINOVITCH, M. & M.J. DE STEFANO (1973b).
Macrophage spreading in vitro. I. Inducers of spreading.
Exp. Cell Res. 77: 323-334.

RAFF, M. (1976).
Self-regulation of membrane receptors.
Nature 259: 265-266.

REAVEN, A.P. & S.G. AXLINE (1973).
Subplasmalemmal microfilaments and microtubules in resting and phagocytozing cultivated macrophages.
J. Cell Biol. 59: 12-27.

REMINGTON, J.S., J.L. KRAHENBUHL & J.W. MENDENHALL (1972).
A role for activated macrophages in resistance to infection with Toxoplasma.
Infect. Immunity 6: 829-834.

REMINGTON, J.S., J.L. KRAHENBUHL & J.B. HIBBS, Jr. (1975).
A role for macrophages in resistance to tumour development and tumour destruction. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 869-891.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

REMOLD, H.G. & J.R. DAVID (1971).
Further studies on migration inhibitory factor (MIF). Evidence for its glycoprotein nature.
J. Immunol. 107: 1090-1098.

REMOLD, H.G. (1973).
Requirement for α-L-fucose on the macrophage membrane receptor for MIF.
J. exp. Med. 138: 1065-1076.

REMOLD, H.G. (1974).

The enhancement of MIF activity by inhibition of macrophage associated esterases.

J. Immunol. 112: 1571-1577.

REMOLD, H.G. & R.D. ROSENBERG (1974).

Enhancement of migration inhibitory factor (MIF) activity by plasma esterase inhibitors.

Fed. Proc. 33: 745 (abst.)

REYNOLDS, H.Y., R.E. THOMPSON & H.B. DEVLIN (1974).

Development of cellular and humoral immunity in the respiratory tract of rabbits to Pseudomonas lipopolysaccharide.

J. clin. Invest. 53: 1351-1358.

REYNOLDS, H.Y., J.P. ATKINSON, H.H. NEWBALL & M.M. FRANK (1975).

Receptors for immunoglobulins and complement on human alveolar macrophages.

J. Immunol. 114: 1813-1819.

RHODES, J. (1975).

Macrophage heterogeneity in receptor activity. The activation of macrophage Fc receptor function in vivo and in vitro.

J. Immunol. 114: 976-981.

RILEY, J.F. (1963).

Functional significance of histamine and heparin in tissue mast cells.

Ann. N.Y. Acad. Sci. 103: 151-163.

RISTER, M. & R.L. BAEHNER (1975).
A comparative study of superoxide dismutase activity in polymorphonuclear leukocytes, monocytes and alveolar macrophages of the guinea pig.
J. Cell. Physiol. 87: 345-355.

ROBERTSON, A.J., N.R. PEDEN, J.H.B. SAUNDERS, J.H. GIBBS, R.C. POTTS, R.A. BROWN, K.G. WORMSLEY & J. SWANSON BECK (1979).
Cimetidine and the immune response.
Lancet ii: 420-421.

ROCKLIN, R. (1976).
Modulation of cellular-immune responses in vivo and in vitro by histamine receptor bearing lymphocytes.
J. clin. Invest. 57: 1051-1058.

RODBELL, M., L. BIRNBAUMER, S.L. POHL & F. SUNDBY (1971).
The reaction of glucagon with its receptor: evidence for discrete regions of activity and binding in the glucagon molecule.
Proc. natn. Acad. Sci. U.S.A. 68: 909-913.

ROMEO, D., G. ZABUCCHI, M.R. SORANZO & F. ROSSI (1971).
Macrophage metabolism: activation of NADPH oxidation by macrophages.
Biochem. biophys. Res. Commun. 45: 1056-1062.

ROMEO, D., G. ZABUCCHI & F. ROSSI (1973a).
Reversible metabolic stimulation of polymorphonuclear leukocytes and macrophages by Concanavalin A.
Nature (New Biol.) 243: 111-112.

ROMEO, D., G. ZABUCCHI, T. MARZI & F. ROSSI (1973b).
Kinetic and enzymatic features of metabolic stimulation of alveolar and peritoneal macrophages challenged with bacteria.
Exp. Cell Res. 78: 423-432.

ROMEO, D., R. CRAMER, T. MARZI, M.R. SORANZO, G. ZABUCCHI & F. ROSSI (1973c).
Peroxidase activity of alveolar and peritoneal macrophages.
J. Reticuloend. Soc. 13: 399-409.

ROOT, R.K. & J.A. METCALF (1977).
H_2O_2 release from human granulocytes during phagocytosis: relationship to superoxide anion formation and cellular catabolism of H_2O_2. Studies with normal and cytochalasin B-treated cells.
J. clin. Invest. 60: 1266-1279.

ROSENSTREICH, D.L. & J.J. OPPENHEIM (1976).
The role of macrophages in the activation of T and B lymphocytes in vitro. In: Immunobiology of the Macrophage; p. 161.
D.S. Nelson (ed.); Academic Press, New York.

ROSENTHAL, A.S., J.T. BLAKE, J.J. ELLNER, D.K. GREINEDER & P.E. LIPSKY (1976).
Macrophage function in antigen recognition by T lymphocytes.
In: Immunobiology of the Macrophage; 131-160.
D.S. Nelson (ed.); Academic Press, New York.

ROSSI, F., G. ZABUCCHI & D. ROMEO (1975).
Metabolism of phagocytosing mononuclear phagocytes at rest and during phagocytosis. In: Mononuclear Phagocytes in Immunity, Infection and Pathology; p. 420.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

ROSZKOWSKI, W., M. PLAUT & L.M. LICHTENSTEIN (1977).
Selective display of histamine receptors on lymphocytes.
Science 195: 683-685.

SABIN, F.R. (1939).
Cellular reactions to a dye-protein with a concept of the mechanism of antibody formation.
J. exp. Med. 70: 67-82.

SALVIN, S.B., S. SELL & J. NISHIO (1971).
Activity in vitro of lymphocytes and macrophages in delayed hypersensitivity.
J. Immunol. 107: 655-662.

SAXON, A., D. MORLEDGE & B. BONAVIDA (1977).
Histamine receptor leukocytes. Organ and lymphoid subpopulation distribution in man.
Clin. exp. Immunol. 28: 394-399.

SCHAYER, R.W. (1959).
Catabolism of physiological quantities of histamine in vivo.
Phys. Rev. 39: 116-126.

SCHAYER, R.W. (1965).
Histamine and circulatory homeostasis.
Fed. Proc. 24: 1295-1297.

SCHMIDT-GAYK, H.E., K.H. JAKOBS & E. HACKENTHAL (1975).
Cyclic AMP and phagocytosis in alveolar macrophages. Influence of hormones and dibutyril cyclic AMP.
J. Reticuloend. Soc. 17: 251-261.

SCHORLEMMER, H.U., P. DAVIES & A.C. ALLISON (1976).
Ability of activated complement components to induce lysosomal enzyme release from macrophages.
Nature 261: 48-49.

SCHORLEMMER, H.U. & A.C. ALLISON (1976).
Effects of activated complement components on enzyme secretion by macrophages.
Immunology 31: 781-788.

SCHROIT, A.J., B. GEIGER & R. GALLILY (1973).
The capacity of macrophage components to inhibit anti-macrophage serum activity.
Eur. J. Immunol. 3: 354-359.

SEEGER, R.C. & J.J. OPPENHEIM (1970).
Synergistic interaction of macrophages and lymphocytes in antigen-induced transformation of lymphocytes.
J. exp. Med. 132: 44-65.

SEEGER, R.C. & J.J. OPPENHEIM (1972).
Macrophage-bound antigens. II. Comparison of the immunogenecity of antigens bound to macrophages, lymphocytes, thymocytes and hepatomic cells.
J. Immunol. 109: 255-261.

SELJELID, R. (1975).
Cytotoxic effect of macrophages on mouse red cells.
In: Mononuclear Phagocytes in Immunity, Infection and Pathology; 911-925.
R. Van Furth (ed.); Blackwell Scientific Publications, Oxford, London, Edinburgh.

SELVARAJ, R.J. & A.J. SBARRA (1967).
The role of the phagocyte in host parasite interaction. VII. Di- and triphosphopyridine nucleotide kinetics during phagocytosis.
Biochim. Biophys. Acta 141: 243-249.

SENIOR, R.M., D.R. BIELEFELD & J.J. JEFFREY (1972).
Collagenolytic activity in alveolar macrophages.
Clin. Res. 20: 88.

SEYBERTH, H.W., H. SCHMIDT-GAYK, K. JAKOBS & E. HACKENTHAL (1973).

Cyclic adenosine monophosphate in phagocytozing granulocytes and alveolar macrophages.

J. Cell Biol. 57: 567-571.

SHORTMAN, K. & J. PALMER (1971).

The requirement for macrophages in the in vitro immune response.

Cell. Immunol. 2: 399-410.

SHURIN, S.B. & T.P. STOSSEL (1978).

Complement (C3)-activated phagocytosis by lung macrophages.

J. Immunol. 120: 1305-1312.

SIMCHOWITZ, L. & I. SPILBERG (1979).

Evidence for the role of superoxide radicals in neutrophil-mediated cytotoxicity.

Immunology 37: 301-309.

SIMON, H.B. & J.N. SHEAGREN (1971).

Cellular immunity in vitro. I. Immunologically mediated enhancement of macrophage bactericidal capacity.

J. exp. Med. 133: 1377-1389.

SIMON, H.B. & J.N. SHEAGREN (1972).

Migration inhibitory factor and macrophage bactericidal function.

Infect. Immunity 6: 101-103.

SINGER, S.J. & G.L. NICOLSON (1972).

The fluid mosaic model of the structure of cell membranes.

Science 175: 720-731.

SINGER, S.J. (1974).
Molecular biology of cellular membranes with application to immunology.
Adv. Immunol. 19: 1-66.

SNYDERMAN, R., H.S. SHIN & M.S. HAUSMAN (1971).
A chemotactic factor for mononuclear leukocytes.
Proc. Soc. Exp. Biol. Med. 138: 387-390.

SNYDERMAN, R., L.C. ALTMAN, M.S. HAUSMAN & S.E. MERGENHAGEN (1972).
Human mononuclear leukocyte chemotaxis. A quantitative assay for humoral and cellular chemotactic factors.
J. Immunol. 108: 857-860.

SODERLAND, S.C. & Y. NAUM (1973).
Growth of pulmonary alveolar macrophages *in vitro*.
Nature 245: 150-152.

SOROKIN, S.P. & J.D. BRAIN (1975).
Pathways of clearance in mouse lungs exposed to iron oxide aerosols.
Anat. Rec. 181: 581-600.

SPRITZER, A.A., J.A. WATSON, J.A. AULD & M. GUETTHOFF (1968).
Pulmonary macrophage clearance. The hourly rates of transfer of pulmonary macrophages to the oropharynx of the rat.
Arch. Environ. Hlth. 17: 726-730.

STEWART, J., D.G. JONES & A.B. KAY (1979).
Metabolic studies on the uptake of ^{14}C-histidine and ^{14}C-histamine and histamine synthesis by guinea pig basophils, *in vitro*.
Immunology 36: 539-548.

STOSSEL, T.P., F. MURAD, R.J. MASON & M. VAUGHAN (1970).
Regulation of glycogen metabolism in polymorphonuclear leukocytes.
J. Biol. Chem. 245: 6228-6234.

STOSSEL, T.P., R.J. MASON & M. VAUGHAN (1971).
A simple sensitive assay for the rate of phagocytosis.
Clin. Res. 19: 468.

STOSSEL, T.P. (1974).
Phagocytosis.
New Eng. J. Med. 290: 717-723.

STOSSEL, T.P. & J.H. HARTWIG (1975).
Interactions between actin, myosin and a new actin-binding protein of rabbit alveolar macrophages. Macrophage myosin Mg^2 adenosine triphosphate requires a cofactor for activation by actin.
J. Biol. Chem. 250: 5706-5712.

STOSSEL, T.P. (1976).
The mechanism of phagocytosis.
J. Reticuloend. Soc. 19: 237-245.

STOSSEL, T.P. & J.H. HARTWIG (1976).
Interactions of actin, myosin and a new acting-binding protein of rabbit pulmonary macrophages. II. Role in cytoplasmic movement and phagocytosis.
J. Cell Biol. 68: 602-619.

TAKANAKA, K. & P.J. O'BRIEN (1975).
Mechanism of H_2O_2 formation by leukocytes. Evidence for a plasma membrane location.
Arch. Biochem. Biophys. 169: 428-435.

TALALAY, P., W.H. FISHMAN & C. HUGGINS (1946).
Chromogenic substrates. II. Phenolphthalein glucuronic acid as substrate for the assay of glucuronidase activity.
J. Biol. Chem. 166: 757-772.

THRASHER, S.G., P. BIGAZZI & S. COHEN (1973a).
Immunofluorescent localization of surface components of guinea pig alveolar macrophages.
Fed. Proc. 32: 977 (abst.)

THRASHER, S.G., T. YOSHIDA, C.J. VAN OSS, S. COHEN & N.R. ROSE (1973b).
Alteration of macrophage interfacial tension by supernatants of antigen-activated lymphocyte culture.
J. Immunol. 110: 321-326.

TSAN, M.F. & R.D. BERLIN (1971a).
Membrane transport in the rabbit alveolar macrophage. The specificity and characteristics of amino acid transport system.
Biochim. Biophys. Acta 241: 155-169.

TSAN, M.F. & R.D. BERLIN (1971b).
Effect of phagocytosis on membrane transport of non-electrolytes.
J. exp. Med. 134: 1016-1035.

TURKER, R.K. (1973).
Presence of histamine H2 receptors in the guinea pig pulmonary vascular bed.
Pharmacology 9: 306-311.

TURNBULL, L.W. & KAY, A.B. (1976).
Eosinophils and mediators of anaphylaxis. Histamine and imidazole acetic acid as chemotactic agents for human eosinophil leucocytes.
Immunology 31: 797-802.

TURNBULL, L.S., TURNBULL, L.W., LEITCH, A.G., CROFTON, J.W. & KAY, A.B. (1977).
Mediators of immediate-type hypersensitivity in sputum from patients with chronic bronchitis and asthma.
Lancet ii: 526-529.

UCHIDA, M. & K. TAKAGI (1977).
Purification of histamine receptors. III. Characterization of receptor rich membrane fraction of small intestinal smooth muscle of the cat.
Japan J. Pharmacol. 27: 1-7.

UNANUE, E.R. (1968).
Properties and some use of antimacrophage antibodies.
Nature 218: 36-38.

UNANUE, E.R. & B.A. ASKONAS (1968a).
The immune response of mice to antigen in macrophages.
Immunology 15: 287-296.

UNANUE, E.R. & B.A. ASKONAS (1968b).
Persistence of immunogenicity of antigen after uptake by macrophages.
J. exp. Med. 127: 915-926.

UNANUE, E.R., J.C. CEROTTINI & M. BEDFORD (1969).
Persistence of the antigen on the surface of macrophages.
Nature 222: 1193-1195.

UNANUE, E.R. (1972).
The regulatory role of macrophages in antigenic stimulation.
Adv. Immunol. 15: 95-165.

UNANUE, E.R. (1978).
The regulation of lymphocyte functions by the macrophage.
Immunol. Rev. 40: 227-255.

UNKELESS, J.C., GORDON, S. & REICH, E. (1974).
Secretion of plasminogen activator by stimulated macrophages.
J. exp. Med. 139: 834-850.

UKENA, T.E. & R.D. BERLIN (1972).
Effect of colchicine and vinblastine on the topographical separation of membrane functions.
J. exp. Med. 136: 1-7.

UHR, J.W. & J.M. PHILLIPS (1966).
In vitro sensitization of phagocytes and lymphocytes by antigen-antibody complexes.
Ann. N.Y. Acad. Sci. 129: 793-798.

VAN FURTH, R., Z.A. COHN, J.G. HIRSCH, W.G. SPECTOR & H.L. LANGEVOORT (1972).
The mononuclear phagocyte system: a new classification of macrophages, monocytes and their precursor cells.
Bull. Wld. Hlth. Org. 46: 845-852.

VAN OSS, C.J. & C.G. GILLMAN (1972).
Phagocytosis as a surface phenomenon. I. Contact angles and phagocytosis of non-opsonized bacteria.
J. Reticuloend. Soc. 12: 283-292.

VAN OSS, C.J., C.F. GILLMAN & A.W. NEUMANN (1974).
Phagocytosis as a surface phenomenon. IV. The minimum size and composition of antigen-antibody complexes that can become phagocytized.
Immunol. Comm. 3: 77-84.

VAN WAARDE, D., E. HULSING HESSELINK & R. VAN FURTH (1978).
Humoral control of monocytopoeisis by an activator and inhibitor.
Agents Action 8: 432-437.

VELO, G.P. & W.G. SPECTOR (1973).

The origin and turnover of alveolar macrophages in experimental pneumonia.

J. Path. 109: 7-19.

VIJEYARATNAM, G.S. & B. CORRIN (1972).

Origin of the pulmonary alveolar macrophage studied in the iprindole-treated rat.

J. Path. 108: 115-118.

VIRCHOW, R. VON (1847).

Die pathologischen Pigmente.

Arch. Pathol. Anat. Phys. Klin. Med. I: 379-486.

VIROLAINEN, M. (1968).

Hematopoeitic origin of macrophages as studied by chromosome markers in mice.

J. exp. Med. 127: 943-951.

VOGT, M.T., C. THOMAS, C.L. VASALLO, R.E. BASFORD & J.B.L. GEE (1971).

Glutathione-dependent peroxidative metabolism in the alveolar macrophage.

J. clin. Invest. 50: 401-410.

VON INS, A. (1876).

Experimentalle Untersuchungen über Kieselstaubinhalation.

Arch. Exptl. Pathol. Pharmakol. V: 169-194.

VON KNAUFF, A. (1867).

Das Pigment der Respirationsorpane.

Arch. Pathol. Anat. Physiol. Klin. Med. XXXIX: 442-475.

WAHL, L.M., S.M. WAHL, S.E. MERGENHAGEN & G.R. MARTIN (1974).

Collagenase production by endotoxin-activated macrophages.

Proc. natn. Acad. Sci. U.S.A. 71: 3598-3601.

WAHL, S.M., J.M. WILTON, D.L. ROSENSTREICH & J.J. OPPENHEIM (1975).

The role of macrophages in the production of lymphokines by T and B lymphocytes.

J. Immunol. 114: 1296-1301.

WALKER, W.S. (1976).

Functional heterogeneity of macrophages. In: Immunobiology of the Macrophage; 91-110.

D.S. Nelson (ed.); Academic Press, New York.

WARD, P.A. (1968).

Chemotaxis of mononuclear cells.

J. exp. Med. 128: 1201-1221.

WARD, P.A., H.G. REMOLD & J.R. DAVID (1969).

Leukotactic factor produced by sensitized lymphocytes.

Science 163: 1079-1081.

WARD, P.A., H.G. REMOLD & J.R. DAVID (1970).

The production by antigen stimulated lymphocytes of a leukotactic factor distinct from migration inhibitory factor.

Cell. Immunol. 1: 162-174.

WARR, G.A. & R.R. MARTIN (1973).

In vitro migration of human alveolar macrophages: effects of cigarette smoking.

Infect. Immunity 8: 222-227.

WEENING, R.S., R. WEVER & D. ROOS (1975).

Quantitative aspects of the production of superoxide radicals by phagocytizing human granulocytes.

J. Lab. clin. Med. 85: 245-252.

WEINSTEIN, Y., K.L. MELMON, H.R. BOURNE & M. SELA (1973).
Specific leukocyte receptors for small endogenous hormones. Detection by cell binding to insolubilized hormone preparations.
J. clin. Invest. 52: 1349-1361.

WEINSTEIN, Y., T. POON, H.R. BOURNE & K.L. MELMON (1975).
A new class of drug conjugates of vasoactive amines to complex or simple carriers has pharmacologic activity.
Clin. Res. 23: 385A.

WEISSMANN, G., R.B. ZURIER, P.J. SPIELER & I.M. GOLDSTEIN (1971a).
Mechanisms of lysosomal enzyme release from leukocytes exposed to immune complexes and other particles.
J. exp. Med. 134: 149s-165s.

WEISSMANN, G., R.B. ZURIER & S. HOFFSTEIN (1971b).
Effect of cyclic AMP on release of lysosomal enzymes from phagocytes.
Nature (New Biol.) 231: 131-135.

WEISSMANN, G., P. DUKOR & G. SESSA (1971c).
Studies on lysosomes: mechanisms of enzyme release from endocytic cells and a model for latency in vitro.
In: Immunopathology of Inflammation; 107-117.
B.K. Fascher & J.C. Houck (eds.); Excerpta Medica, Amsterdam.

WEISSMANN, G., R.B. ZURIER & S. HOFFSTEIN (1972).
Leukocyte proteases and the immunologic release of lysosomal enzymes.
Am. J. Path. 68: 539-559.

WEISSMANN, G., R.B. ZURIER & S. HOFFSTEIN (1973).
Leukocytes as secretory organs of inflammation.
Agents Action 3: 370-379.

WEISSMANN, G., I. GOLDSTEIN, S. HOFFSTEIN, G. CHAUVETAUD & R. ROBINEAUX (1975a).
Yin/Yang modulation of lysosomal enzyme release from polymorphonuclear leukocytes by cyclic nucleotides.
Ann. N.Y. Acad. Sci. 256: 222-232.

WEISSMANN, G., I. GOLDSTEIN, S. HOFFSTEIN & P.K. TSUNG (1975b).
Reciprocal effects of cAMP and cGMP on microtubule dependent release of lysosomal enzymes.
Ann. N.Y. Acad. Sci. 253: 750-762.

WEST, J., D.J. MORTON, V. ESMANN & R.L. STJERNHOLM (1968).
Carbohydrate metabolism in leukocytes. VII. Metabolic activities of the macrophage.
Arch. Biochem. Biophys. 124: 85-90.

WILKINSON, P.C. (1972).
Characterization of the chemotactic activity of casein for neutrophil leukocytes and macrophages.
Experientia 28: 1051-1052.

WILKINSON, P.C., G.J. O'NEILL & K.G. WAPSHAW (1973).
Role of anaerobic coryneforms in specific and non-specific immunological reactions. III. Production of a chemotactic factor specific for macrophages.
Immunology 24: 997-1006.

WILKINSON, P.C. (1975).
Leukocyte locomotion and chemotaxis. The influence of divalent cations and cation ionophores.
Exp. Cell Res. 93: 420-426.

WILKINSON, P.C. (1976).
Chemotaxis: cellular and molecular aspects. In: Immunobiology of the Macrophage; 349-365.
D.S. Nelson (ed.); Academic Press, New York.

WING, E.J., I.D. GARDNER, F.W. RYNING & J.S. REMINGTON (1977).
Dissociation of effector functions in populations of activated macrophages.
Nature 268: 642-644.

WOOD, D.D. & S.L. GAUL (1974).
Enhancement of the humoral response of T cell-dependent murine spleens by a factor derived from human monocytes in vitro.
J. Immunol. 113: 925-933.

WROBLEWSKY, F. & J.S. LaDUE (1955).
Lactic dehydrogenase activity in blood.
Proc. Soc. Exp. Biol. Med. 90: 210-213.

WYATT, H.V., H.R. COLTEN & T. BORSOS (1972).
Production of the second (C2) and fourth (C4) components of guinea pig complement by single peritoneal cells: Evidence that one cell may produce both components.
J. Immunol. 108: 1609-1614.

YAM, L.T., C.Y. LI & W.H. CROSBY (1971).
Cytochemical identification of monocytes and granulocytes.
Am. J. clin. Path. 55: 283-290.

ZEIGER, R.S., D.L. YURDIN & H.R. COLTEN (1976).
Histamine metabolism. II. Cellular and subcellular localization of the catabolic enzymes, histaminase and histamine methyl transferase in human leukocytes.
J. Allergy clin. Immunol. 58: 172-179.

ZEMBALA, M., W. PTAK & M. HANCZAKOWSKA (1973).
Macrophage and lymphocyte co-operation in target cell distinction *in vitro*.
Clin. exp. Immunol. 15: 461-465.

ZIGMOND, S.H. & J.G. HIRSCH (1972).
Effect of cytochalasin B on polymorphonuclear leukocyte locomotion, phagocytosis and glycolysis.
Exp. Cell Res. 73: 383-393.

ZURIER, R.B., S. HOFFSTEIN & G. WEISSMANN (1973a).
Cytochalasin B: effect on lysosomal enzyme release from human leukocytes.
Proc. natn. Acad. Sci. U.S.A. 70: 944-848.

ZURIER, R.B., S. HOFFSTEIN & G. WEISSMANN (1973b).
Mechanism of lysosomal enzyme release from human leukocytes. I. Effect of cyclic nucleotide and colchicine.
J. Cell Biol. 58: 27-41.

ZURIER, R.B., G. WEISSMANN, S. HOFFSTEIN, S. KAMMERMAN & H.H. TAI (1974).
Mechanisms of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP autonomic agonists and agents which affect microtubule function.
J. clin. Invest. 53: 297-309.

Clin. exp. Immunol. (1979) 35, 462–469.

Histamine receptors on guinea-pig alveolar macrophages: chemical specificity and the effects of H1- and H2-receptor agonists and antagonists

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SUMMARY

Various guinea-pig leucocytes were tested for their capacity to bind histamine coupled as a rabbit serum albumin conjugate (H-RSA) to formalised ox red cells. The percentage of rosette-forming target cells was directly related to the concentration of erythrocyte-bound H-RSA. Under optimal experimental conditions the numbers of rosettes varied from 60 to 81% for alveolar macrophages, 14 to 73% for peritoneal macrophages, 14 to 30% for blood monocytes, 27 to 48% for lymph node cells, 7 to 24% for blood lymphocytes and 0 to 29% for peritoneal and blood neutrophils. Virtually no histamine rosettes were formed with eosinophils or basophils.

Free histamine partially inhibited rosette formation by alveolar macrophages in a dose-dependent fashion from 10^{-3} to 10^{-5} mol/l, and complete inhibition was achieved by the H-RSA conjugate. In contrast, amines closely related to histamine such as L-histidine and the major histamine catabolites, imidazoleacetic acid, 1,4-methylhistamine, 1-methyl-4-imidazoleacetic acid and N-acetylhistamine, had no inhibitory effect.

The histamine H1–receptor antagonists, mepyramine and chlorpheniramine, and the H1-receptor agonist, 2– (2–aminoethyl) thiazole, all inhibited rosette formation by alveolar macrophages in a dose-dependent fashion. However, the H2-receptor antagonists, burimamide and metiamide, and the H2-receptor agonists, Dimaprit and 4–methyl–histamine, were inactive.

These experiments suggest that (1) compared to other leucocytes, histamine receptors are particularly well expressed on the alveolar macrophage, (2) these receptors have a high degree of specificity for histamine in that other amines, closely related chemically, did not inhibit rosette formation, and (3) the binding of histamine to the alveolar macrophage membrane is H1- and not H2- receptor dependent.

INTRODUCTION

It has previously been shown that most human peripheral blood leucocytes, but not erythrocytes, adhered to Sepharose beads coated with a histamine-rabbit serum albumin conjugate, suggesting the presence of surface receptors for this amine (Melmon *et al.*, 1972; Weinstein *et al.*, 1973). Using the same conjugate, but with red cells in place of Sepharose beads, Kedar & Bonavida (1974) were able to develop a rosette technique for demonstrating histamine receptors on mouse leucocytes from a variety of lymphoid tissues. Subsequently, the same group reported that human monocytes and a proportion of peripheral blood T and B lymphocytes also expressed receptors for histamine (Saxon, Morledge & Bonavida, 1977).

The release of histamine and other pharmacological agents from sensitized lung fragments following challenge with specific antigen is well known. The lung is a major site for histamine storage and high

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concentrations of histamine have been found in the sputum in pulmonary disorders associated with immediate-type hypersensitivity (Turnbull *et al.*, 1977; Turnbull *et al.*, 1978). For these reasons it seemed important to establish the presence of histamine receptors on the alveolar macrophage, as well as other leucocytes associated with biological properties of histamine, such as the neutrophil, eosinophil and basophil. For instance, histamine was shown to increase chemokinesis of neutrophils (Anderson, Glover & Rabson, 1977), to promote directional migration of eosinophils (Clark, Gallin & Kaplan, 1975; Turnbull & Kay, 1976; Jones & Kay, 1977), and to be synthesized and formed in the basophil (Galli *et al.*, 1976; Stewart, Jones & Kay, 1979). By rosette-inhibition experiments we have also examined the chemical specificity of binding by histamine-coated red cells, as well as the effects of H1- and H2-receptor agonists and antagonists.

MATERIALS AND METHODS

Materials were obtained as follows. Histamine dihydrochloride, rabbit serum albumin, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (ECDI) and L-histidine (Sigma, London); HEPES (Wellcome Laboratories, Beckenham, Kent); mepyramine maleate (May & Baker Ltd., Dagenham, Essex); chlorpheniramine (Allen & Hanburys Ltd., London); imidazoleacetic acid hydrochloride (ImAA), 1, 4-methylhistamine dihydrochloride (1, 4-MeHm), N-acetylhistamine and 1-methyl-4-imidazoleacetic acid hydrochloride (1, 4-MeImAA) (Calbiochem, California, USA); sodium diatrizoate (45% Hypaque, Winthrop, Surbiton-on-Thames, Surrey); Ficoll (Pharmacia, London); burimamide, metiamide, 4-methylhistamine dihydrochloride (4-MeHm), 2-(2-aminoethyl) thiazole dihydrochloride (2-2-AET) and S [3-(N, N dimethylamino) propyl] isothiourea (Dimaprit) were a gift from Smith, Kline & French Laboratories Limited, Welwyn Garden City, Hert. All other reagents were B.D.H. (Poole, Dorset) Analar grade.

Preparation of leucocytes. Dunkin Hartley strain guinea-pigs of either sex and weighing 250–400 g were used throughout.

(*a*) *Alveolar macrophages.* These were obtained by tracheo-bronchial lavage (Myrvik, Leake & Farris, 1961) using HEPES buffered saline, pH 7·3–7·4, from guinea-pigs anaesthetized with intraperitoneal sodium pentobarbital (40 mg/kg). The macrophages were enriched by density centrifugation on Ficoll–Hypaque (d = 1·08 g/ml) (Böyum, 1968) at 650 *g* for 30 min at 4°C, washed twice in phosphate buffered saline (PBS), pH 7·2, and brought to a final concentration of 5×10^6 cells/ml in PBS. Such preparations usually contained between 85 and 95% macrophages.

(*b*) *Peritoneal macrophages and eosinophils.* These were obtained by lavage of normal guinea-pigs using 20 ml PBS containing heparin (4 u/ml). They were used either unpurified, or following density centrifugation on Ficoll–Hypaque as described above.

(*c*) *Peritoneal neutrophils.* These were harvested by lavage as described above, 3 hr following the intraperitoneal administration of 20 ml glycogen (1·0 mg/ml) in PBS.

(*d*) *Blood monocytes, neutrophils, lymphocytes and eosinophils.* These were obtained by puncture of the abdominal aorta and collected into heparinised tubes (5 u/ml). Erythrocytes were sedimented in 3% gelatin for 15 min at room temperature. The leucocyte-rich supernatant was removed, washed three times in PBS and the cell counts again adjusted in PBS to a final concentration of 5×10^6/ml.

(*e*) *Lymph node cells.* These were collected from the paratracheal lymph nodes by gently teasing with a scalpel and filtering the resultant cell suspension through a stainless steel sieve (120 wires per inch). The cells were then washed twice in BBS and adjusted to a final concentration of 5×10^6/ml.

(*f*) *Bone marrow basophils.* These were obtained from the humeri, tibiae and fibulae of guinea-pigs given whole sheep's blood (1 : 1 with Alsever's solution) on 12 successive days as described previously (Dvorak *et al.*, 1974).

If any of the preparations described above contained unacceptable numbers of contaminating erythrocytes, these were lysed by suspending the cells briefly in 0·82% ammonium chloride, washing several times in PBS and readjusting the cell counts to 5×10^6/ml.

Preparation of histamine-rabbit serum albumin conjugate (H-RSA). The H-RSA conjugate was prepared using the procedure described by Kedar & Bonavida (1974). Briefly, 1·4 g histamine dihydrochloride, 200 mg rabbit serum albumin (RSA) and 1·2 g ECDI were dissolved in 20 ml of PBS, pH 7·2. After incubation for 1 hr at room temperature with intermittent shaking, the solution was dialysed at 4°C against 5 litres of PBS which was changed three times over a 48 hr period. The controls, which were prepared in parallel under the same conditions, were (1) RSA_{ECDI} : 200 mg of RSA and 1·2 g of ECDI in 20 ml of PBS, and (2) RSA_u : 200 mg of RSA in 20 ml of PBS without the coupling reagent. These preparations were stored at −20°C and used for up to 10 weeks after preparation.

Coupling of H-RSA to ox red cells. Ox red cells were washed three times in PBS and adjusted to a 50% suspension with 0·2% formalsaline. After 20 min at room temperature, the cells were washed three times in PBS. 2·5 ml of various dilutions (usually 1 : 60—see Fig. 1) of H-RSA (or RSA_{ECDI} or RSA_u) were mixed with 0·25 ml of formalised ox cells in PBS and 20 mg of ECDI in 1·0 ml of PBS and incubated for 45 min at room temperature with intermittent agitation. The red cells were then washed three times in PBS and adjusted to a final concentration of $2·5 \times 10^8$ cells/ml.

Rosette assay. Duplicate 0·1 ml volumes of leucocyte suspensions (5×10^6/ml) were mixed with an equal volume of red cells ($2·5 \times 10^8$/ml), centrifuged at 500 *g* for 5 min at 4°C and incubated (usually for 15 min—see Fig. 2) at 0°C or,

where necessary, at 37°C. The resultant cell pellets were resuspended by gentle agitation and 0·2 ml of 2% formalsaline was added. Smears were made in duplicate from each suspension. Alveolar macrophages were stained with either May Grunwald–Giemsa or the non-specific esterase technique described by Yam, Li & Crosby (1971). Other cell types were identified by the May Grunwald–Giemsa stain. The percentage of rosettes (those cells binding three or more erythrocytes) was then counted in 200 random leucocytes, areas of cell clumping being excluded.

Inhibition of rosette formation was studied as follows: 0·1 ml volumes of suspensions containing alveolar macrophages were centrifuged at 500 *g* for 5 min at 0°C and the supernatants discarded. The test agents, freshly prepared, or the PBS controls, were added to the cell pellets and incubated for 15 min at 37°C or 0°C, prior to the addition of the red cell suspension. The samples were then treated as described above. The effect of the test agents on cell viability was assessed by trypan blue exclusion before and after treatment.

The percentage inhibition of rosette formation was calculated as $\left[\frac{a-b}{a}\right] \times 100$, where a and b represent the percentage of leucocyte rosettes in preparations pre-incubated with PBS or drug, respectively.

RESULTS

The histamine rosette assay

The effect of varying the amount of H-RSA coupled to ox red cells on rosette formation by guinea-pig alveolar macrophages is shown in Fig. 1. The percentage of rosettes was directly related to the

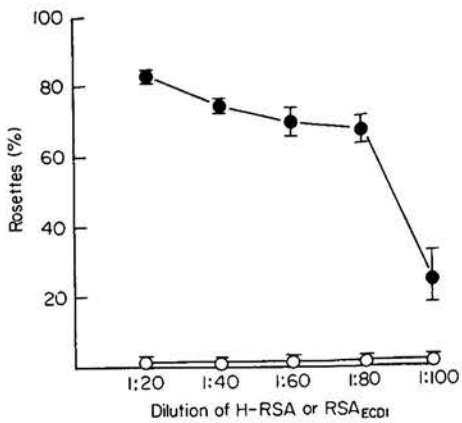

FIG. 1. The effect of dilutions of H-RSA (●) or RSA$_{ECDI}$ (○) on rosette formation by alveolar macrophages. Each point represents the mean ± s.e. of three experiments.

amount of H-RSA coupled, whereas no rosette formation was seen when the same dilutions of the control, RSA $_{ECDI}$, were added to the indicator erythrocytes. Also, although not shown in Fig. 1, RSA$_u$ plus ox cells and the ox cells alone failed to form rosettes. There was little difference in the percentage of rosettes between the 1 : 20 and 1 : 80 dilutions of H-RSA but binding was considerably less with a 1 : 100 dilution. In general, concentrations of H-RSA greater than 1 : 20 produced an unacceptable degree of lysis of the indicator ox red cells.

The time course of rosette formation using ox cells coupled with three concentrations of H-RSA is shown in Fig. 2. Rosette formation was virtually instantaneous with only a small increase being observed from 0 to 30 min with all dilutions. At 1:60, the numbers of rosettes were greater at 0, 5 and 15 min when compared to the other dilutions, but at 30 min they were virtually identical to the 1:80 dilution. Since the 1 : 60 dilution showed a slight increase in rosette formation with time up to 15 min, this concentration and incubation time were used in all further experiments.

Free histamine inhibited rosette formation, both at 0°C and 37°C, in a dose-dependent fashion from 10^{-3} to 10^{-5} mol/l, although only 44 and 48% inhibition was achieved with the highest concentrations (Table 1). In contrast, the H-RSA conjugate completely inhibited rosette formation at a 1:60 dilution, whereas equivalent amounts of RSA $_{ECDI}$ and RSA$_u$ gave no inhibition when compared to the diluent (PBS) control.

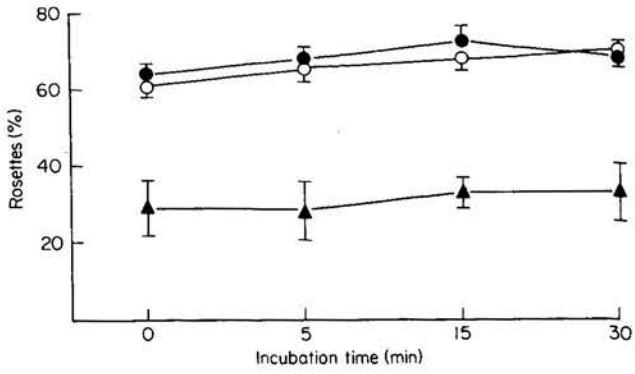

FIG. 2. The effect of incubation time on rosette formation by alveolar macrophages with red cells coated with H-RSA dilutions of 1:60 (●), 1:80 (○) and 1:100 (▲). Each point is the mean±s.e. of three experiments.

Histamine rosettes by various guinea-pig leucocytes

The capacity of mononuclear phagocytes, lymphocytes, neutrophils, eosinophils and basophils to form histamine rosettes is shown in Fig. 3. Mononuclear phagocytes were obtained from three sources: lung washings, the peritoneum and peripheral blood. In general, alveolar macrophages formed greater numbers of histamine rosettes than all of the other cell types tested, including peritoneal macrophages. Rosette formation did not appear to depend on the degree of enrichment of cells—at least not by those leucocytes in which this was examined, i.e. alveolar and peritoneal macrophages, lymph node cells and eosinophils. The ranges of the percentage of histamine rosettes for the different cell types were alveolar macrophages, 60–81%; peritoneal macrophages, 14–73%; blood monocytes, 14–30%; lymph node cells, 27–48%; blood lymphocytes, 7–24%; peritoneal and blood neutrophils, 0–26% and 0–29%, respectively. Virtually no histamine rosettes were formed by eosinophils or basophils.

Inhibition of rosettes by histamine and related compounds

The capacity of L-histidine, histamine and major histamine catabolites to inhibit histamine rosette formation by alveolar macrophages is shown in Table 2. Significant dose-dependent inhibition of rosette formation was achieved by histamine, from 10^{-3} to 10^{-5} mol/l, but not by L-histidine or the major histamine catabolites, ImAA, 1, 4-MeHm, N-AcHm and 1, 4-MeImAA, when the experiments were performed either at 0°C and 37°C.

TABLE 1. Inhibition of rosette formation with alveolar macrophages by histamine, H-RSA, RSA_{ECDI} and RSA_u*

Pre-treatment	Number of experiments	Percentage of rosettes ± 1 s.e. (percentage inhibition)	
		0°C	37°C
PBS	12	62±2	58±3
Histamine: 10^{-3} mol/l	12	35±2 (44)	30±2 (48)
10^{-4} mol/l	12	42±2 (32)	38±2 (35)
10^{-5} mol/l	12	49±2 (21)	46±3 (21)
PBS	3	66±0	67±3
H-RSA 1:60	3	1±1 (98)	1±1 (99)
RSA_{ECDI} 1:60	3	66±2 (0)	69±4 (0)
RSA_u 1:60	3	70±1 (0)	71±1 (0)

* Key for abbreviations in the Materials and Methods section.

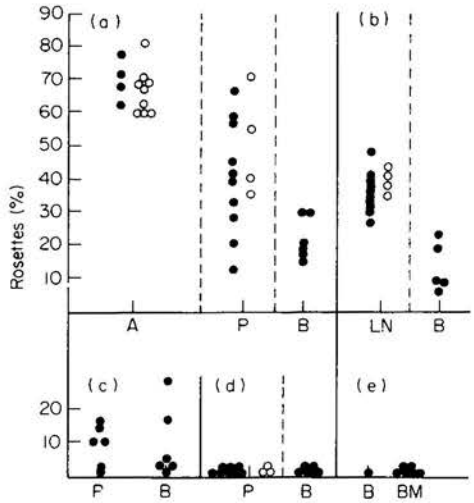

FIG. 3. Histamine rosettes by (a) mononuclear phagocytes, (b) lymphocytes, (c) neutrophils, (d) eosinophils and (e) basophils. In some experiments cells were unfractionated (●) or enriched (○) by centrifugation on coll-Hypaque. A = alveolar, P = peritoneal, B = blood, LN = lymph node, BM = bone marrow.

TABLE 2. Inhibition of rosette formation with alveolar macrophages by histamine, L-histidine, ImAA, 1,4-MeImAA 1,4-MeHm and N-AcHm at 0°C or 37°C

	Percentage inhibition of rosette formation					
	10^{-3} mol/l		10^{-4} mol/l		10^{-5} mol/l	
Drug added	0°C	37°C	0°C	37°C	0°C	37°C
Histamine	45±3*	45±3	32±3	34±2	19±2	20±2
L-histidine	5±2	3±1	1±1	1±1	4±2	1±1
ImAA	13±7	7±3	10±7	3±3	4±3	4±2
1,4-MeHm	1±1	7±6	1±1	0±0	3±3	2±2
N-AcHm	1±1	2±2	2±1	1±1	2±1	0±0
1,4-MeImAA	0±0	1±1	4±3	0±0	3±2	0±0

* The results are the mean±s.e. of four experiments with the exception of histamine (twelve experiments).

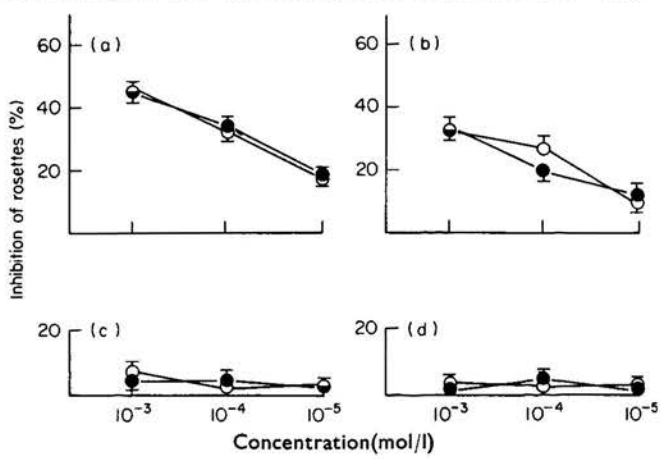

FIG. 4. Inhibition of rosette formation with alveolar macrophages by H1- and H2-receptor agonists (a) histamine, (b) 2-2-AET, (c) 4-MeHm and (d) Dimaprit at 0°C (○) or 37°C (●). Each point represents the mean±s.e. of three experiments.

The effect of H1- and H2-receptor agonists and antagonists

The H1 agonist, 2-2-AET, inhibited the binding of H-RSA conjugated red cells to alveolar macrophages in a dose-dependent fashion, from 10^{-3} to 10^{-5} mol/l with a degree of inhibition only slightly less than that achieved by histamine (Fig. 4). In contrast, the H2 agonists, 4-MeHm and Dimaprit, had no inhibitory effect under the same conditions. Similarly, inhibition of rosettes was demonstrable with the H1 antagonists, mepyramine and chlorpheniramine, at concentrations from 10^{-4} to 10^{-6} mol/l at 0°C and 37°C, but not by the H2 antagonists, burimamide and metiamide, under the same conditions (Fig. 5).

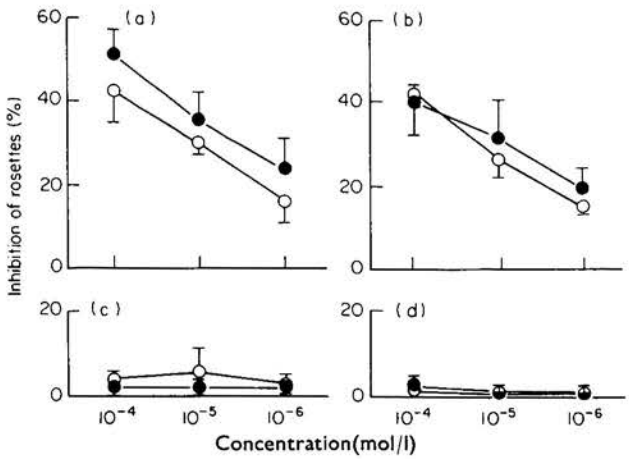

FIG. 5. Inhibition of rosette formation with alveolar macrophages by H1- and H2-receptor antagonists (a) mepyramine, (b) chlorpheniramine, (c) burimamide and (d) metiamide at 0°C (○) or 37°C (●). Each point represents the mean ± s.e. of three experiments.

DISCUSSION

The technique described here for coupling histamine to erythrocytes has been modified from previously described methods (Kedar & Bonavida, 1974; Saxon *et al.*, 1977). We found that, in general, ox cells were less susceptible than sheep cells to lysis by the various chemical agents employed. Also, formalised ox erythrocytes were more stable in this respect than untreated cells. In addition, we found that with 20 mg/ml ECDI the degree of haemolysis was consistently less than with the dose (40 mg/ml) recommended in the original technique for use with sheep cells (Kedar & Bonavida, 1974; Saxon *et al.*, 1977).

Histamine rosettes were formed almost instantaneously (Fig. 2) and no significant differences were observed at 0°C and 37°C (Tables 1 and 2, Figs 4 and 5). In these respects, the histamine receptor differs from those for complement (C3b, C3d and C4) and IgG (Fc) on human granulocytes since complement receptors were expressed better at 37°C and Fc receptors at 0°C and maximal rosette formation was achieved at 10 min (Wong & Wilson, 1975; Anwar & Kay, 1977).

We are unable to say with certainty that the cell membrane of the various leucocytes studied binds free histamine, as opposed to histamine bound as a conjugate. However, the receptor is apparently specific for the histamine moiety of the conjugate in that substances closely related chemically, such as L-histidine and the major histamine catabolites, imidazoleacetic acid, 1, 4-methylhistamine, N-acetylhistamine, and 1, 4-methyl-imidazoleacetic acid, did not inhibit the reaction to any significant extent, whereas free histamine inhibited binding by 45% at 10^{-3} mol/l (Table 2). Furthermore, rabbit serum albumin treated with the coupling reagent (RSA_{ECDI}), RSA_u and untreated ox cells were also without effect, whereas the histamine conjugate completely inhibited binding of ox cells coupled to H-RSA.

If stabilization of histamine is required for optimal recognition then this may have biological significance in that the binding of histamine to plasma proteins has been demonstrated by a number of workers (Parrot, Urquia & Laborde, 1952; Guirgis, 1967; Freeman, 1969). Histamine-binding (or 'histaminopexy') is apparently non-covalent since histamine can be easily dissociated from plasma proteins. Proteins with histamine-binding activity have been identified in α_1, α_2, β and γ-globulins (Guirgis, 1967; Freeman, 1969).

The present experiments suggest that histamine rosettes are formed entirely at H1-receptor sites since the binding was inhibited in a dose-dependent fashion by H1 antagonists including mepyramine and chlorpheniramine, whereas the H2 antagonists, burimamide and metiamide, had no effect. Furthermore, the reaction was similarly blocked by the histamine H1-receptor agonist, 2-2-AET (Durant, Ganellin & Parsons, 1975), but not by the H2 agonists, 4-MeHm (Durant *et al.*, 1975) and Dimaprit (Parsons *et al.*, 1977). In this respect, our observations differ from other histamine–dependent properties of leucocytes. For instance, the histamine-induced inhibition of lysosomal enzyme release from phagocytosing human neutrophils (Busse & Sosman, 1976) and increase in neutrophil chemokinesis (Anderson *et al.*, 1977) appears to be mediated by H2–receptors as was inhibition, by histamine, of macrophage migration inhibition factor release from sensitized lymphocytes challenged with antigen (Rocklin, 1976) and T lymphocyte cytolysis (Plaut, Lichtenstein & Henney, 1973). However, our findings are in agreement with the original description of histamine rosette formation which was shown to be inhibited by the classical antihistamines (H1 antagonists) which included diphenhydramine, tripelennamine, antazoline and pyrilamine (Melmon *et al*, 1972).

Under the experimental conditions described, guinea-pig peritoneal macrophages and blood monocytes expressed fewer histamine receptors than alveolar macrophages. The reasons for this are unclear but it would be of interest to examine mononuclear phagocytes from other sources to determine whether this really is a special property of lung macrophages.

The finding that only 30–40% of lymph node cells formed histamine rosettes is in agreement with the observations of Saxon *et al.* (1977) who found that a similar proportion of the total lymphocyte population bound histamine coupled to red cells and that the receptors were expressed in both the T and B subpopulations. The possible importance of histamine-bearing lymphoid cells in immune tolerance or suppression has been discussed elsewhere (Melmon *et al.*, 1975).

Of particular interest was the apparent inability of eosinophils and basophils to form histamine rosettes since these cells are known to be associated with various histamine-related biological events. For instance, basophils synthesize and release this amine (Galli *et al.*, 1976; Stewart *et al.*, 1979) and the anaphylactic release of histamine from basophils was inhibited by histamine itself; an effect that was mediated by an H2-receptor-dependent mechanism (Lichtenstein & Gillespie, 1973). Although certain cell membranes clearly recognise histamine in order to enable its participation in these histamine-dependent functions, this does not necessarily mean that these recognition units can be visualized directly by a procedure such as the rosette technique described here. Similarly, eosinophils, which respond in directional locomotion to histamine (Clark *et al.*, 1975; Turnbull & Kay, 1976; Jones & Kay, 1977) and also presumably have a membrane recognition unit for histamine-induced chemotaxis, were unable to bind directly with stabilized histamine under the conditions described here.

We cannot exclude the possibility that the covalent coupling of histamine to albumin somehow precludes, possibly by steric hindrance, binding of histamine to H2 receptors. This may explain our inability to demonstrate rosette formation with eosinophils and basophils. For instance, histamine-induced eosinophil locomotion was shown to be predominantly H2-dependent (Clark *et al.*, 1975; Jones & Kay, 1977) as was the inhibition of histamine release, by histamine, from basophils (Lichtenstein & Gillespie, 1973).

The precise role of the histamine receptor which we and others have demonstrated by the rosette technique is unclear, although it may serve as a useful marker for the classification of subpopulations of certain mononuclear phagocytes, lymphocytes and possibily neutrophils.

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REFERENCES

ANDERSON, R., GLOVER, A. & RABSON, A.R. (1977) The *in vitro* effects of histamine and metiamide on neutrophil motility and their relationship to intracellular cyclic nucleotide levels. *J. Immunol.* **118**, 1690.

ANWAR, A.R.E. & KAY, A.B. (1977) Membrane receptors for IgG and complement (C4, C3b and C3d) on human eosinophils and neutrophils and their relation to eosinophilia. *J. Immunol.* **119**, 976.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97, 77.

BUSSE, W.W. & SOSMAN, J. (1976) Histamine inhibition of neutrophil lysosomal enzyme release: an H2 histamine receptor response. *Science*, **194**, 737.

CLARK, R.A.F., GALLIN, J.I. & KAPLAN, A.P. (1975) The selective eosinophil chemotactic activity of histamine. *J. exp. Med.* **142**, 1462.

DURANT, G.J., GANELLIN, C.R. & PARSONS, M.E. (1975) Chemical differentiation of histamine H1- and H2-receptor agonists. *J. Med. Chem.* **18**, 905.

DVORAK, H.F., SELVAGGIO, S.S., DVORAK, A.M., COLVIN, R.B., LEAN, D.B. & RYPYSC, J. (1974) Purification of basophilic leucocytes from guinea pig blood and bone marrow. *J. Immunol.* **113**, 1694.

FREEMAN, M.L. (1969) Histamine binding in serum of children with asthma. *Aust. J. Paediat.* **5**, 133.

GALLI, S.J., GALLI, A.S., DVORAK, A.M. & DVORAK, H.F. (1976) Metabolic studies of guinea pig basophilic leucocytes in short-term tissue culture. I. Measurement of histamine-synthesizing capacity by using an isotopic thin layer chromatographic assay. *J. Immunol.* **117**, 1085.

GUIRGIS, H.M. (1967) Separation of a histamine-binding fraction from normal human serum. *Int. Arch. Allergy Appl. Immunol.* **31**, 587.

JONES, D.G. & KAY, A.B. (1977) Chemotactic activity of guinea pig eosinophils for the ECF-A acidic tetrapeptides, histamine, histamine metabolites, and the effect of H1- and H2-receptor antagonists. *Int. Arch. Allergy Appl. Immunol.* **55**, 277.

KEDAR, E. & BONAVIDA, B. (1974) Histamine receptor-bearing leukocytes (HRL). I. Detection of histamine receptor-bearing cells by rosette formation with histamine-coated erythrocytes. *J. Immunol.* **113**, 1544.

LICHTENSTEIN, L.M. & GILLESPIE, E. (1973) Inhibition of histamine release by histamine controlled by H2-receptor. *Nature (Lond.)*, **244**, 287.

MELMON, K.L., BOURNE, H.R., WEINSTEIN, J. & SELA, M. (1972) Receptors for histamine can be detected on the surface of selected leukocytes. *Science*, **177**, 707.

MELMON, K.L., WEINSTEIN, Y., SHEARER, G.M., POON, T., KRASNY, L. & SEGAL, S. (1975) Isolation of cells with specific receptors for amines: opportunities and problems. *Cell Membrane Receptors for Viruses, Antigens, and Antibodies, Polypeptide Hormones and Small Molecules* (ed. by R. F. Beers and E. G. Bassett), Chap. 10, p. 117. Raven Press, New York.

MYRVIK, Q.N., LEAKE, E.S. & FARRIS, B. (1961) Studies on pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a high state of purity. *J. Immunol.* **86**, 128.

PARROT, J.-L., URQUIA, D.-A. & LABORDE, C. (1952) Action histaminopexique de serum humain et son pouvoir protecteur a l'egard de l'histamine. Modification par la dialyse ou par l'addition d'histamine. *Compt. Rend. Soc. Biol.* **146**, 1052.

PARSONS, M.E., OWEN, D.A.A., GANELLIN, C.R. & DURANT, G.J. (1977) Dimaprit—]S-]3-(N,N-dimethylamino) propyl] isothiourea]—a highly specific histamine H2-receptor agonist. Part 1. Pharmacology. *Agents and Actions*, **7**, 31.

PLAUT, M., LICHTENSTEIN, L.M. & HENNEY, C. (1973) Increase in histamine receptors on thymus-derived effector lymphocytes during the primary immune response to alloantigens. *Nature (Lond.)*, **244**, 284.

ROCKLIN, R.E. (1976) Modulation of cellular immune responses *in vivo* and *in vitro* by histamine receptor-bearing lymphocytes. *J. clin. Invest.* **57**, 1051.

SAXON, A., MORLEDGE, V.D. & BONAVIDA, B. (1977) Histamine-receptor leucocytes (HRL). Organ and lymphoid subpopulation distribution in man. *Clin. exp. Immunol.* **28**, 394.

STEWART, J., JONES, D.G. & KAY, A.B. (1979) Metabolic studies on the uptake of ^{14}C-histidine and ^{14}C-histamine and histamine synthesis by guinea pig basophils, *in vitro*. *Immunology* (in press).

TURNBULL, L.W. & KAY, A.B. (1976) Eosinophils and mediators of anaphylaxis. Histamine and imidazoleacetic acid as chemotactic agents for human eosinophil leucocytes. *Immunology*, **31**, 797.

TURNBULL, L.W., TURNBULL, L.S., CROFTON, J.W. & KAY, A.B. (1978) Variations in chemical mediators of hypersensitivity in the sputum of chronic bronchitics: correlation with peak expiratory flow. *Lancet*, **ii**, 184.

TURNBULL, L.S., TURNBULL, L.W., LEITCH, A.G., CROFTON, J.W. & KAY, A.B. (1977) Mediators of immediate-type hypersensitivity in sputum from patients with chronic bronchitis and asthma. *Lancet*, **ii**, 526.

WEINSTEIN, Y., MELMON, K.L., BOURNE, H.R. & SELA, M. (1973) Specific leukocyte receptors for small endogenous hormones. Detection by cell binding to insolubilized hormone preparations. *J. clin. Invest.* **52**, 1349.

WONG, L. & WILSON, J.D. (1975) The identification of Fc and C3 receptors on human neutrophils. *J. Immunol. Methods*, **7**, 69.

YAM, L.T., LI, C.Y. & CROSBY, W.H. (1971) Cytochemical identification of monocytes and granulocytes. *Amer. J. clin. Path.* **55**, 283.